Interactions of *Fusarium virguliforme* with Other Common Soybean Root Pathogens and the Soybean Aphid

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DEDICATION

To my loving parents, Rick and Lisa, who taught me that with love and patience, anything is possible.

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ABSTRACT

Many diseases reduce soybean (*Glycine max*) yield every year, with root diseases often having the greatest impacts on yield in the Midwestern U.S. One important root pathogen of soybean is *Fusarium virguliforme* (*Fv*), the causal agent of sudden death syndrome (SDS). Even though soybean plants displaying SDS symptoms are commonly attacked by multiple fungal pathogens and insects, it is unknown how interactions between *Fv* and other attackers may alter SDS disease development and soybean growth. In our first study, we examined potential interactions between *Fv* and other common soybean root pathogens including *F. acuminatum, F. oxysporum, F. solani,* and *Clonostachys rosea*. Interactions were assessed in vitro and in soybean plants to evaluate their effect on SDS disease development and soybean growth. Results from the in vitro studies suggest that the growth of *Fv* was not affected by any of these fungi, but the growth of *F. acuminatum* and *F. oxysporum* isolates was reduced in the presence of *Fv*. Further results suggest that coinfection of soybean with *Fv* and *C. rosea* and *Fv* and *F. solani* can reduce SDS foliar disease severity and that coinfection of soybean with *Fv* and *F. oxysporum* and *Fv* and *F. acuminatum* can increase SDS foliar severity compared to the *Fv*-only treatment under growth chamber conditions. Coinfections of soybean with *Fv* and all eight fungal test isolates individually did not increase or decrease levels of root rot or plant biomass compared to infection by *Fv* alone. In our second study, we examined potential interactions between *Fv* and the soybean aphid, *Aphis glycines,* in greenhouse, growth chamber, and field experiments to evaluate their effect on SDS disease development, aphid growth, and soybean growth. Overall, the results suggest that *Fv* and soybean aphids have minimal to no interaction with one another while co-occurring on soybean when SDS develops to low

levels, as was the case in these studies. There was no clear effect of soybean aphid herbivory on SDS foliar or root disease development in these studies nor did we detect a clear effect of combined soybean aphid herbivory and *Fv* infection on soybean growth. To our knowledge this is the first investigation into potential interactions between *Fv* and other common root pathogens and *Fv* and the soybean aphid.

LITERATURE REVIEW

Soybean Production

Soybean (*Glycine max* (L.) Merr.) is a legume crop originating from East Asia and one of the most important crops grown worldwide[1]. In 2018, 4.54 billion bushels of soybean were produced in the United States[2] with 389 million bushels produced in Minnesota alone[3]. Soybeans are harvested for their meal and oil. Roughly 70% of all soybeans produced in the United States are fed to livestock, with poultry being the largest consumer, 15% are used for human consumption, and 5% used for biodiesel production. The remainder of soybean produced in the U.S is used for a variety of industrial uses such as paints, cleaners, and plastics[4]. Although soybean is a well-suited crop for large areas of the United States, multiple biotic (especially diseases and insects) and abiotic stresses reduce production every year.

Significant soybean diseases

In the United States, soybean production is constrained by many foliar and root diseases. The occurrence of soybean diseases varies annually by location and is influenced by many factors, including environmental conditions, host resistance, production practices, and cropping and disease history[5,6]. From 2010 to 2013, the top five yield-limiting diseases in the northern United States and Ontario, Canada included soybean cyst nematode (SCN) (*Heterodera glycines* Ichinohe), seedling diseases (caused by *Rhizoctonia, Pythium, Fusarium,* and *Phytophthora* spp.)*, Phytophthora* root and stem rot, sudden death syndrome (SDS), and charcoal rot[5]. In 2010 and 2014, when weather was favorable for SDS, it was the second and third most destructive soybean disease, respectively[5,7]. Sudden

death syndrome was among the top five most destructive soybean diseases for all years between 2010-2014, highlighting its importance in soybean production in Northern United States and Ontario, Canada[5].

History of sudden death syndrome

Sudden death syndrome was first discovered in Arkansas in 1971 by H.J. Walters and has since spread to most soybean producing states[8]. In 1984 SDS was identified in Mississippi, Missouri, Kentucky, and Tennessee, and by 1986 SDS appeared in Illinois and Indiana[8]. Since then, the distribution of SDS has increased to include Georgia[8], Iowa[9], Kansas[10], Louisiana[11], Michigan[12], Nebraska[13], New York[14], North Dakota[15], Ohio[8], Pennsylvania[16], South Dakota[17], Wisconsin[18], Ontario, Canada[19], and Minnesota[20].

In 2006 and 2007, SDS was confirmed in 21 Minnesota counties all located north of 45 ° N from the east to west borders of the state. Efforts to map the distribution of SDS in Minnesota have led to the identification of 38 counties (Fig. 1.1) with confirmed SDS in soybean ranging as far north as Otter Tail county (D. Malvick, personal communication, October 2019). The distribution of SDS is likely to continue to increase throughout the upper Midwest, including Minnesota, resulting in new risks to soybean growers in the northern soybean-producing areas.

Outside of the United States and Canada, SDS has also been reported in the South American countries of Argentina, Brazil, Paraguay, Bolivia, and Uruguay[21]. In more recent years, surveys conducted in 2013 and 2014 detected SDS for the first time in South Africa[22] and in Malaysia in 2014[23].

SDS biology and disease cycle

The causal organism of SDS was initially reported as *Fusarium solani* f. sp. *glycines*[24]. Since then, the nomenclature has been revised, and phylogenetic and morphological studies have identified seven distinct *Fusarium* spp. that cause SDS on soybean. Within clade II of the *Fusarium solani* species, *F. azuicola*[25], *F. brasiliense*[21], *F. crassistipitatum*[26], *F. cuneirostrum*[21], *F. tucumaniae*[27], *F. virguliforme*[27], and one un-named *Fusarium* species in South Africa[22] have been reported to cause SDS on soybean. Although *F. brasiliense, F. cuneirostrum,* and *F. virguliforme* have been documented in the United States[21], the only species found there causing SDS in soybean has been *Fusarium virguliforme* O'Donnell & T. Aoki (*Fv*) until recently. In 2019, *F. brasiliense* was first reported to cause SDS in soybean in Michigan[28]. However, the primary causal agent of SDS and most prominent SDS-causing *Fusarium* species remains *Fv* in the United States. In Minnesota the only confirmed causal agent of SDS to date is *Fv.*

Yield losses to soybean resulting from SDS range from slight to 100% on individual plants[8,29]. These losses are a result of two phases of disease development. First, *Fv* colonizes soybean root tissues during the spring causing root rot and crown necrosis (Fig. 1.2). Second, *Fv* secretes toxins through the xylem leading to interveinal chlorosis and necrosis of foliar tissues[30] (Fig. 1.2). This phase typically occurs late in the season during soybean reproductive stages and can lead to premature defoliation and pod abortion[31]. Although *Fv* causes foliar symptoms, the pathogen remains only in the roots and lower stems of plants and has not been isolated from leaves (Fig. 1.2).

SDS disease management

Sudden death syndrome management relies primarily on the use of genetic disease resistance [32] and the use of seed treatment fungicides[33,34]. These may be complemented by the use of cultural practices including management of soybean cyst nematode [35–37], crop rotation[38], tillage[39], drainage, and in some cases delayed planting[40]. However, there are no complete sources of resistance to SDS and cultural methods yield inconsistent results.

Resistance. Planting resistant cultivars is the most effective tool that soybean growers have to manage SDS. Unfortunately, breeding for SDS resistance is challenging due to the quantitative nature of disease resistance, large environmental influences on disease development, and imperfect screening methods[40,41]. Genetic resistance to SDS is governed by multiple quantitative trait loci (QTL), with each QTL providing a small contribution to resistance[42]. Further, SDS foliar severity has a low correlation with root rot severity, suggesting that resistance to foliar and root disease is independent[41,42]. Currently, there are no commercial varieties with complete resistance to SDS, but there are many that show different levels of partial resistance to the disease in the field[33,40]. The search for effective and durable resistance to SDS continues to be a priority in the development of SDS management tools for growers.

Fungicidal seed treatments. Fungicides are used to complement host resistance to SDS or are used when SDS resistance is not available in selected varieties. Although many commercially available fungicides are registered for soybean, few are effective in managing SDS. Several of these fungicides were tested prior to 2000 as seed treatments for their ability to control SDS disease and none were found to reduce root rot caused by *Fv*[43]. The first fungicide discovered and marketed that showed strong efficacy against *Fv*

was fluopyram (ILeVO; BASF) and was registered as a seed treatment for SDS in December 2014. Fluopyram is effective against *Fv* in vitro[34] and reduces SDS while increasing soybean yields in field studies[33]. In September 2019, adepidyn (Saltro; Sygenta). was registered as a seed treatment for SDS. Preliminary field trials with adepidyn have indicated that it is effective for managing SDS, but further work is needed to confirm these results (D. Malvick, personal communication, October 2019). Both fluopyram and adepidyn are classified as succinate dehydrogenase inhibitor (SDHI) fungicides and act by blocking a crucial enzyme involved in respiration, the mitochondrial enzyme succinate dehydrogenase[44].

Interactions between soybean cyst nematode and SDS. SDS and soybean cyst nematode (SCN), *Heterodera glycines,* are commonly found in the same fields[8]. Often the most severe outbreaks of SDS are found in fields where high levels of SCN are also present,[8,37] suggesting that *Fv* and SCN may act synergistically to enhance SDS development[45,46]. In field microplot studies, a disease-increasing interaction between SCN and *Fv* has been documented[35–37]. However, other studies have found no detectable interaction between SCN and SDS severity[47,48]. The inconsistency of results regarding the relationship between SCN and *Fv* and the yield losses associated with both pathogens warrants additional research.

Tillage. Many pathogens, including *Fv*, can survive on crop residues and can be carried over as inoculum for subsequent years. Generally speaking, reduced tillage or no till practices increase the risk of disease from soil borne pathogens because these practices leave pathogen infested crop residues on the surface, a primary source of pathogen inoculum[49]. Removal of crop residues and/or burying them through tillage may reduce SDS

disease severity[39], but results are inconsistent and conflicting. A study in Missouri reported that SDS foliar incidence was higher in no-till fields compared to disk-till or ridge-till[50]. However, the impact that tillage has on soil compaction and moisture may play a more important role in managing SDS[39,51–53]. In one study, the effects that subsoiling with deep tillage had on soil compaction, moisture, and the severity of SDS was examined and results showed that subsoiling increased soil porosity, reduced soil moisture, and could significantly reduce SDS foliar severity[54]. In addition, another study reported a negative correlation between SDS severity and soil macro-porosity, suggesting that increasing soil water drainage through tillage could aid in disease suppression[55].

Crop rotation. Crop rotation can significantly reduce the incidence and severity of diseases caused by some soilborne pathogens[56]. Crop rotation with a non-host allows time for some pathogen populations to decline in the soil and for the degradation of crop residues needed for some pathogens to survive. In some cases, merely rotating away from a host crop for an ample amount of time can result in a decrease in the viability of pathogen survival structures[49]. The current research on the use of crop rotation to control SDS, however, is inconsistent[57–59]. This suggests that *Fv* survives longer or has a broader host range than originally thought. One of the earliest reports limited the host range of *Fv* to soybean, mung bean, green bean, lima bean, and cowpea[8]. Since then, an additional ten symptomatic (alfalfa, pinot bean, navy bean, pea, white clover, Canadian milk vetch, sugar beet, and canola) and five asymptomatic (corn, wheat, ryegrass, pigweed, and lambsquarters) hosts of *Fv* have been reported[38]. Additionally, *Fv* has been shown to survive long-term in corn residue[60]. These findings could provide an explanation for why crop rotation has not been successful in reducing levels of SDS in fields. Therefore,

determining the host range *Fv* has been a crucial step in implementing crop rotation as a disease management tool for SDS.

Planting date. Commonly, when growers plant early in the spring, increases in SDS disease severity are seen as a result of wet soils. Root rot symptoms due to *Fv* colonization are more severe in soils with high moisture[40,45,54,61], which is likely why SDS epidemic years begin with wet spring weather. Although if the rest of the growing season is dry, planting in wet soils may not result in high levels of SDS disease[7]. By planting later, soils may become drier, and therefore less favorable for *Fv* germination and colonization of root tissues. However, it is not typically recommended for growers to plant later due to a risk of yield reduction[29].

Interactions between *Fusarium* spp. and soybean root pathogens

In addition to being attacked by *Fv*, other pathogenic soilborne fungi and oomycetes including *Pythium, Phytophthora, Rhizoctonia,* and other *Fusarium* spp. are often found infecting soybean roots[62,63]. Because of the frequency of their isolation and ability to infect soybean, understanding the interactions between *Fv* and these fungal genera would be valuable information for implementing disease management strategies. However, only one study to date has investigated interactions with *Fv* specifically. Under greenhouse conditions, an experiment was conducted to determine if the presence of both *Fv* and *Fusarium graminearum* (*Fg*) resulted in an increased negative impact on soybean growth compared with each pathogen alone[64]. Interestingly, results showed that there was no evidence for a negative impact of the *Fv* + *Fg* treatment compared with *Fv* and *Fg* alone[64]. In addition, a few studies have reported interactions occurring between other *Fusarium* spp. (not *Fv*) with *Pythium* and *Rhizoctonia* and there are no studies on *Fusarium* and

Phytophthora spp. interactions. Synergistic interactions between *Fusarium* and *Pythium* spp. on pea[65], snap bean[66], and peanut[67] were reported. A more recent study, however, found no interactions between *Fusarium* and *Pythium* spp. on seedling disease in soybean[68]. Similarly, the interaction between *F. oxysporum* and *R. solani* on soybean root rot severity was shown to be additive[69], but no interaction was observed between *F. solani* f. sp. *phaseoli*, and *R. solani* on snap beans[66].

Clonostachys rosea is another fungus that is often found inhabiting soybean roots[62] and can be a mycoparasite with a saprophytic life phase[70]. In a Minnesota survey identifying fungal and oomycete species associated with soybean roots in 2007 and 2008, *C. rosea* was among the most frequently isolated species[62]. Historically, *C. rosea* has been of interest due to its potential as a biocontrol agent and has been shown to be a mycoparasite on some *Fusarium* spp[71,72]. In culture, DNA of *F. verticillioides* was reduced in the presence of *C. rosea,* suggesting that *C. rosea* actively destroyed the fungal mycelium of *F. verticillioides*[73]. Additionally, *C. rosea* was found to suppress the sporulation of *F. culmorum* and *F. graminearum* on wheat straw, and of *F. culmorum, F. graminearum, F. proliferatum* and *F. verticillioides* on maize stalks[74]. However, more recently, there have been reports of *C. rosea* being pathogenic on soybean[75] and faba bean[76]. On soybean, a pathogenic *C. rosea* isolate caused taproot necrosis and interveinal chlorosis and marginal necrosis on leaf trifoliates, and successful isolation of the fungus from petioles of symptomatic trifoliates indicated that the fungus colonized the plants systemically[75]. However, the impact of *C. rosea* infection on soybean production is unknown.

Unlike interactions between *Fusarium* and other root pathogens, much more attention has been given to interactions that exist among *Fusarium* spp. This is likely

because many diseases caused by *Fusarium* spp. are disease complexes[77,78]. In a disease complex, different pathogen species are responsible for causing damage on a common host. Pathogens within a disease complex can overlap spatially and geographically and are commonly found coinfecting their hosts[79]. Co-infecting pathogens may affect each other positively (synergism), negatively (antagonism), or not at all (coexistence)[80]. Therefore, pathogen interactions can significantly shape the pathogen community, directly affecting disease dynamics and yield[79].

Interactions among *Fusarium* spp.

Competition between *Fusarium* species that occupy the same niche may result from one of three interactions: competitive exploitation, interference competition, or parasitic fitness[81–83]. Competitive exploitation refers to the ability of one fungus to obtain resources faster than another (i.e. no direct interactions). For example, when grown together in vitro, the biomass of the maize pathogens *Ustilago maydis* and *Fusarium verticillioides* decreased due to the depletion of nutrient resources after an initial period of accelerated growth [84]. One important component affecting the outcome of competitive exploitation is temperature. For example, *F. moniliforme* was able to outcompete *F. graminearum* when coinoculated on maize ears because of its increased ability to grow at a broader range of temperatures compared to the latter[82]. Interference competition involves a chemical or mechanical barrier that limits a competing fungus from obtaining resources from a shared substrate. Although not described for interactions among *Fusarium* spp., other microbes have been reported to affect *Fusarium* spp. through interference competition. For instance, an endophytic *Phoma* sp. colonizing finger millet secretes tenuazonic acid, preventing the growth of pathogenic *F. graminearum*[85]. Interference competition by means of a

mechanical barrier was also demonstrated in finger millet with the bacterial species *Enterobacter,* which forms specialized root hairs that prevent entry of *F. graminearum* and trap and kill the fungus [86]. Lastly, parasitic fitness refers to the aggressiveness of an isolate. This is important for intraspecific competition and has been observed among *Fusarium* species[81,82]. When rye was coinoculated with four distinct isolates of *F. culmorum*, disease severity was reduced compared to when rye was inoculated with only one isolate[81].

Most documented interactions between *Fusarium* species have been shown to be competitive. For example, a negative correlation of *F. moniliforme* with both *F. graminearum* and *F. subglutinans* was documented based on the incidence of the pathogens in corn kernels[87]. In another study performed on *Fusarium* pathogens that cause Fusarium head blight of wheat, it was revealed that different *Fusarium* species likely compete when infecting wheat ears[88]. Competition between *Fusarium* spp. can be host mediated, and the attack by one species can activate host defenses prior to subsequent infections by another. For example, infection of tomato by a nonpathogenic strain of *F. oxysporum* induced host resistance and subsequently reduced colonization of roots by a pathogenic strain of *F. oxysporum*[89].

Interactions between *Fusarium* species can also be synergistic. For example, foot and crown rot of wheat is caused by a complex of *Fusarium* species (*F. graminearum, F. culmorum, F. poae,* and *F. sporotrichioides*) and the presence of *F. graminearum* was found to increase the incidence of the other *Fusarium* species[90]. In a field study, maize ears inoculated with *F. graminearum* were found to facilitate subsequent infection by *F. verticillioides*[91]. Fungal infection can suppress host defenses, making the host more susceptible to subsequent infections. This has been shown in maize where early infection

by *F. verticillioides* suppresses the production of secondary defense metabolites; therefore, aiding in the success of subsequent colonization by several other *Fusarium* species[92].

Pathogenic *Fusarium* on soybean roots

Several pathogenic *Fusarium* spp. are commonly isolated from soybean roots. In a Minnesota survey identifying fungal and oomycete species associated with symptomatic soybeans in 2007 and 2008, the most prevalent *Fusarium* spp. isolated was *F. oxysporum,* followed by *F. solani* and *F. acuminatum*[63]. This agrees with the results from a similar survey conducted in Iowa from 2007-2009[64].

Fusarium acuminatum Ellis & Everhart is a soil saprophyte found in temperate regions across the world[93]. Although generally a saprophyte and secondary invader associated with root, stem, and crown rot diseases on a variety of hosts, some isolates can cause root rot in legume species. Legume hosts affected by *F. acuminatum* include alfalfa, pea, and soybean[63,93–96]. In addition, *F. acuminatum* has been reported as a root pathogen of corn[97], sorghum, wheat[98], barley[99], ginseng[100], pumpkin[101], clover[102], and sugar beet[103].

Fusarium oxysporum Schlechtendahl emend. Snyder & Hansen has the greatest known global distribution among the *Fusarium* spp[93]. The species includes both plant pathogenic and nonpathogenic strains, all of which are commonly found inhabiting soils worldwide[93]. *F. oxysporum* is an important vascular wilt and root and crown rot pathogen of agricultural, garden, and ornamental crops[104] and as a species may have caused more damage to agricultural crops than any other plant pathogen[105]. Pathogenic strains of *F. oxysporum* can have a very narrow host specificity. As a result, two subspecific groups were made within the species, *forma speciales* (group strains based on their host range)

and races (cultivar-level specialization). To date, 106 *formae speciales* have been described within the *F. oxysporum* species[104].

Plant pathogenic strains of *F. oxysporum* can cause root rot and vascular wilting. The latter is the most commonly encountered, and symptoms are a result of *F. oxysporum* colonizing the vascular system leading to yellowing and wilting of the plant [106]. Vascular wilt diseases caused by *F. oxysporum* include Fusarium wilt, Fusarium blight, and Fusarium yellows[104]. *F. oxysporum* can also cause root rot symptoms and these diseases are called basal rot, Fusarium stem, crown, and/or root rots[104].

Fusarium solani (Marius) Appel & Wollenweber emend. Snyder & Hansen is ubiquitous in soil and found worldwide. This species is a complex, comprised of at least 45 phylogenetic and/or biological species based on morphological and molecular traits, and broadly identified as *F. solani*[93]. They are saprophytes commonly found in soil and plant debris and pathogens of economically important host plants. *F. solani* has been documented as a pathogen across a diverse group of host plants including many legumes and tropical plants. Economically important hosts of *F. solani* include alfalfa, bean, pea, soybean, avocado, citrus, cocoyam, cowpea, orchids, passion fruit, squash, pepper, and potato[93]. On soybean, *F. solani* and *F. oxysporum* are common causal agents of Fusarium root rot disease. Symptoms of Fusarium root rot include poor emergence and productivity, stunting, and root rot[107].

Soybean Aphid in the United States

Soybean aphid, *Aphis glycines* Matsumura (Hemiptera: Aphididae), native to Asia, is an invasive pest in North America. Since its first detection in a Wisconsin soybean field in 2000[108], the soybean aphid (SBA) has grown to be the most important insect pest of

soybean throughout the Midwest[109]. By the end of the summer 2000, SBA was reported in 10 states including Wisconsin, Michigan, Minnesota, Iowa, Illinois, Indiana, Ohio, Kentucky, Missouri, and West Virginia. Between 2000 and 2004, SBA spread quickly across the United States and Canada reaching 22 states and 3 Canadian provinces[110–112]. In 2009, the geographic range of SBA expanded even further to include 30 states[113].

Soybean Aphid as an Agricultural Pest

Before the introduction of SBA into the United States, the use of insecticides on soybean fields in the Upper Midwest was infrequent, with less than 0.1% of soybean acreage receiving applications[113]. In comparison, insecticides were applied to 16% of soybean acreage in 2018[114], primarily for soybean aphid management, indicating that the SBA resulted in a dramatic increase in the use of insecticides for soybean production[111].

Infestation symptoms of soybean by SBA include wrinkled and distorted foliage, early defoliation, stunting, and reductions in plant growth, pod size, and seed weight[115]. Soybean injury resulting from SBA infestation includes a reduction in photosynthetic rates[116], vectoring of plant viruses[117,118], and honeydew secretion stimulating sooty mold growth that also results in reduced photosynthesis[119]. Resulting yield losses can reach up to 45% in soybean[120]. However, the impact that SBA has on soybean yield is highly dependent on the developmental stage of soybean[121].

Soybean Aphid life cycle in North America

The soybean aphid has a complex lifecycle characterized as heteroecious (host-alternating) and holocyclic (sexual reproduction during part of lifecycle)[111]. During the spring and summer, SBA feed on soybean and reproduce parthenogenically (asexual

reproduction) producing both winged and wingless female morphs throughout the growing season. Soybean aphid populations can increase exponentially with a doubling time of 6-7 days[120]. Winged offspring (alates) can travel to and colonize other areas within fields and can be dispersed long distances by wind, leading to outbreaks in new areas[122]. Many factors contribute to the production of alates during the summer including temperature, host-quality, crowding, and interactions with natural enemies and mutualists[123,124].

As fall approaches, reductions in photoperiod and temperature influence SBA to produce gynoparae (winged females) who migrate to buckthorn (*Rhamnus* species), also invasive to North America[111]. Here gynoparae give birth to wingless sexual females (oviparae) who are then sought out by winged males from soybean for sexual reproduction. Resulting eggs are deposited underneath buckthorn leaf buds where they overwinter. In the following spring, eggs hatch into apterous, viviparous females and after three to four generations on buckthorn the alataes are produced and migrate to soybean during the host's early growth stages (V1-V5)[125].

Soybean Aphid Management

Integrated pest management (IPM) is a multifaceted and preventative pest management approach that reduces risks from pests using multiple pest management related strategies. As a first line of defense, IPM programs use cultural methods and select resistant cultivars to prevent pests from becoming a threat. Regular scouting of fields is done to monitor pests and determine when pest populations are approaching their economic injury level (EIL), when the cost of control (e.g. insecticide applications) is equal to the yield losses associated with a pest[126]. It is important to act before pest populations reach the EIL, and thus growers are recommended to use control methods preemptively when a

lower pest density, the economic threshold (ET), is reached. The ET for SBA on soybean is 250 aphids per plant and after reaching the ET it takes roughly 7 days before SBA populations will exceed the EIL of 675 aphids per plant[120]. Therefore, closely monitoring fields for SBA is critical for timely and effective management of the pest.

Management of SBA thus relies on preventing populations from reaching damaging levels. To do this, growers use host-plant resistance, promote natural enemy populations, and apply insecticides[127].When host-plant resistance and natural enemies are not sufficient to control SBA populations, broad-spectrum foliar insecticides are used. Organophosphates and pyrethroids and are the most common insecticides used for controlling SBA populations[128]. However, these insecticides are non-specific and kill both SBA and their natural enemies, which can lead to SBA populations rebounding quickly after treatment[129]. In addition, repeated spraying of insecticides can lead to the development of insecticide resistant SBA. Resistance to insecticides in SBA has been documented in Asia and North America[130,131]. Due to the development of insecticide resistance, other management tactics must be incorporated into SBA integrated pest management programs such as biological control and host-resistance.

Biological control can help to suppress SBA populations. The soybean aphid escaped predation from many of its natural enemies in Asia upon its introduction to North America. However, generalist natural enemies including predators, parasitoids, and entomopathogenic (insect-attacking) fungi began to feed on SBA following its introduction to North America and now play an important role in the suppression of SBA[132–134]. Of these enemies, coccinellids and pirate bugs (*Orius insidiosus*) are the main drivers in the biological control of SBA in North America[113]. Additionally, ten species of parasitic wasps

in North America (families: Aphelinidae and Braconidae) were found to parasitize SBA, but these parasitoid species are far less effective than those found in Asia that contribute largely to the suppression of SBA there[113]. Entomopathogenic fungi can also attack SBA, but their suppression of SBA is variable. This is likely because entomopathogenic fungi need a suitable environment for infection[133,135].

Aphid-resistant soybean varieties can directly reduce SBA populations and reduce insecticide use, thus helping to build natural enemy populations. Host plant resistance to aphids reduces host attractiveness (antixenosis), reduces survival and offspring numbers (antibiosis), and/or results in tolerance to infestation[109]. Resistance to SBA depends on single genes called Rag genes, short for resistance to *A. glycines.* Four resistance genes have been identified; *Rag1*[136]*, Rag2*[137], *rag3,* and *rag4*[138]. Since their deployment, biotypes of aphids have been identified that can overcome *Rag1* and *Rag2* resistance[139,140]. Thus, the release of single-gene resistance sources has proven unsustainable. More durable resistance may result by releasing antibiosis and antixenosis resistance in combination[113].

Cross-compartment interactions between herbivores and pathogens

Often, plants are simultaneously attacked by a complex community of biotic stressors, including insect herbivores and pathogens, creating opportunities for attackers to interact. Within the fields of plant pathology and entomology, much attention has been given to pathogen-pathogen and herbivore-herbivore interactions and only more recently to the interactions that may exist between pathogens and herbivores[141]. Interactions between herbivores and pathogens may occur directly, for example, when herbivores fed on fungal mycelia or toxic fungal compounds[142]. Other interactions are indirect and mediated by the host. These interactions typically occur when the pathogen and herbivore

are separated on their host, either spatially or temporally, and can have important consequences for herbivore and pathogen population dynamics and plant yield[143,144].

Even when pathogens and herbivores are separated by the soil surface (cross-compartment), interactions still can occur that are host-mediated. The vascular system of plants allows for long-distance communication between roots and shoots, enabling plants to mount whole-plant defense responses, regulate growth, and allocate resources[145]. The tight linking between roots and shoots allows for cross-compartment interactions between belowground and aboveground attackers through the activation of systemic induced defense responses and changes in host primary metabolism[141,146].

The activation of signaling pathways by pathogen or herbivore leads to systemic and whole-plant defense responses. These responses can directly affect the ability of the host to defend itself locally or "prime" distant plant parts for upcoming attacks[147]. Priming of defense responses can play an important role in mediating cross-compartment interactions between attackers. For instance, root herbivory by *Acalymma vitattum* on cucumber increased the host's defenses against the foliar oomycete pathogen downy mildew[148]. In another example, aboveground herbivory of pepper by the aphid *Myzus persicae* primed systemic defense responses leading to reduced colonization of roots by the bacterial pathogen *Ralstonia solanacearum*[149]. The main hormones involved in controlling local and systemic plant defense responses are salicylic acid (SA), jasmonic acid (JA), and ethylene[150]. In general, the SA pathway is stimulated by and is effective against biotrophic pathogens and piercing-sucking herbivores, while the JA pathway responds to and is effective against necrotrophic pathogens and chewing herbivores[144]. An antagonistic cross-talk between SA and JA pathways has long been speculated[151] with SA

being shown to suppress JA[152], and this cross-talk likely plays an important role in the regulation and fine-tuning of defense responses. Resulting trade-offs in defense responses may then occur when plants are faced with different attackers. For example, infection by the biotrophic pathogen *Hyaloperonospora arabidopsidis* suppressed JA defense responses activated by the leaf herbivore, *Pieris rapae*[153]. Because these pathways are activated by and effective against different groups of organisms, interactions between herbivores and pathogens will likely depend on the specific organisms involved, i.e. insect feeding-guild and pathogen lifestyle[144]. Ultimately, a wide range of defensive traits are activated by systemic induced defense signaling pathways including morphological (e.g. trichomes[154]), production of defensive proteins and toxins (e.g. terpenoid aldehydes in cotton[155]), or release of volatiles (i.e. repel attackers or attract predators of attackers)[147,156].

Herbivore and pathogen attack may also induce responses that alter the host's primary metabolism. Primary metabolites may be used to synthesize secondary defense metabolites, have defensive functions themselves, serve as signals in defensive pathways, or can be reallocated to protect them during attack for subsequent plant regrowth[157]. Therefore, changes in the host's primary metabolism can have profound impacts on cross-compartment interactions. For example, the severity of *Fusarium* crown rot of alfalfa was enhanced by a sap-feeding insect *Spissistilus festinus*. The root carbohydrates concentrations were decreased following feeding by *S. festinus*, which may have led to increased crown rot[158]. On ragwort, aboveground herbivory by the catepillar, *Mamestra brassicae*, caused a decrease in the concentration of pyrrolizidine alkaloids present in roots which led to an increase in soil fungal pathogen populations[159]. In addition, changes in amino acid and sugar concentrations of the phloem can have significant effects on aphid

performance. For example, attack by ectoparasitic and endoparasitic nematodes lowered the amino acid content in phloem sap and reduced aphid fecundity[160]. Similarly, the aphid *Euceraphis betulae* preferred birch leaves infected with the fungus *Marssonina betulae,* and they were larger and displayed enhanced embryo development on the infected leaves[161]. This interaction was likely mediated by the plant since the fungus and aphid occupy different portions of the leaf, and increased concentrations of free amino acids were found in the phloem of infected leaves that may promote aphid growth[161,162].

Pathogen infection can also modify the interactions between herbivores and their natural enemies, although this has not been reported for cross-compartment interactions[156,163,164]. For example, infection of *Brassica rapa* leaves with powdery mildew strongly affected the production of herbivore-induced plant volatiles in response to *Pieris brassicae* feeding, which negatively affected the attraction of its parasitoid wasp *Cotesia glomerate.* This suggested that oviposition and feeding in mildew-infected plants may be a survival strategy for *P. brassicae*[165]. Therefore, a multitrophic approach should be taken when investigating cross-compartment interactions between pathogens and herbivores.

Conclusion

Soybean is an important legume crop in Minnesota in addition to being one of the most important crops grown worldwide. Although soybean is well-suited for large areas of the United States, multiple biotic stresses reduce production every year. In Minnesota, an important yield limiting disease is sudden death syndrome (SDS) caused by the fungus *Fusarium virguliforme* (*Fv*). Despite the fact that soybean plants infected with *Fv* are also challenged simultaneously by other pathogen and insect attackers, little work has been done to explore interactions between *Fv* and other fungal pathogens and herbivores.

Understanding of how *Fv* interacts with other pathogens and herbivores will be important for soybean risk assessment and pest management. The goals of this research were to determine how other fungal root pathogens (*Fusarium* spp. and *Clonostachys rosea)* and the soybean aphid influence SDS disease development and to determine how combined attacks by these organisms and *Fv* may impact soybean growth.

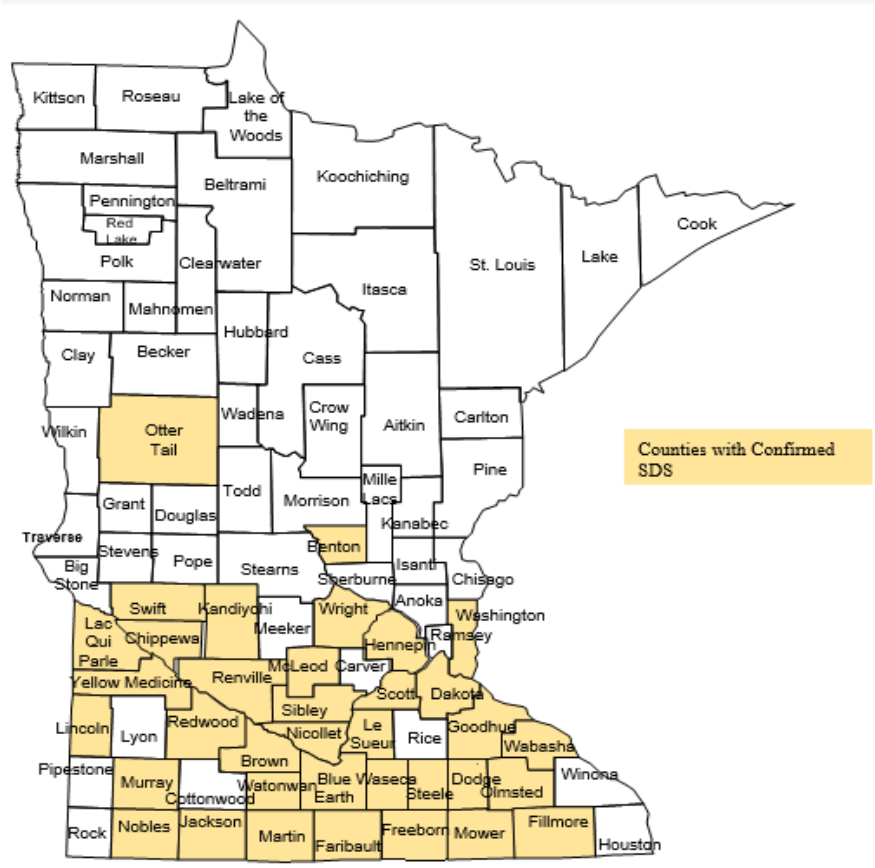

Figure 1.1. Minnesota counties confirmed with sudden death syndrome (SDS) as of 2018 (Personal Communication with D. Malvick, University of Minnesota).

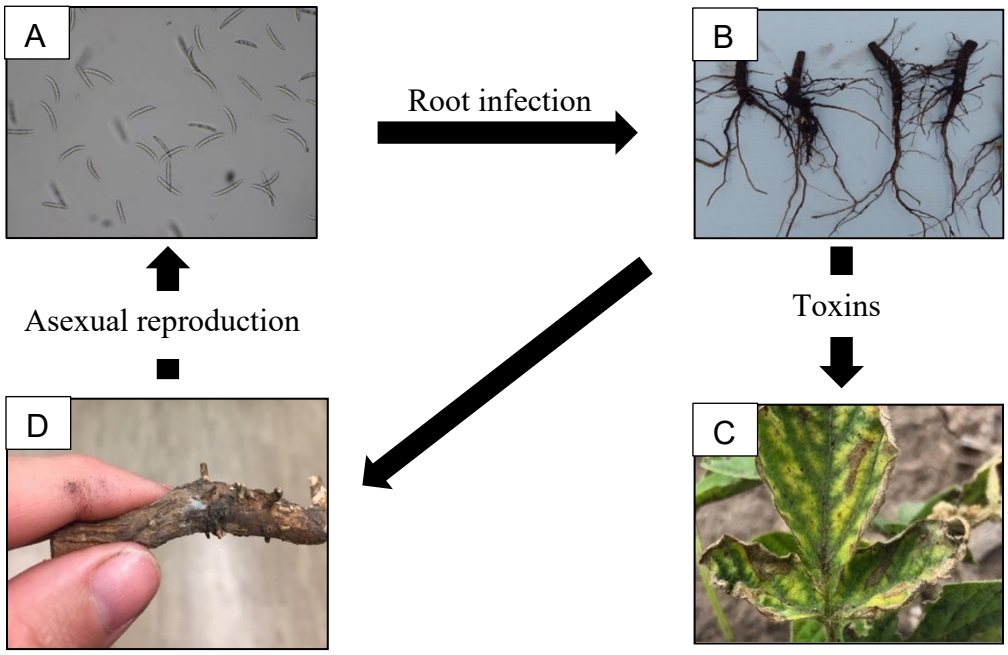

Figure 1.2. Sudden death syndrome disease cycle caused by *Fusarium virguliforme* (*Fv*). *Fv* likely survives in crop residues or freely in the soil as thick-walled chlamydospores. In the spring, chlamydospores germinate to produce conidia (**A**) that initiate infection of soybean roots in the early stages of growth leading to the colonization of root tissues and root rot[52] (**B**). Fungal toxins are translocated through the xylem and induce foliar symptoms[30] (**C**). *Fv* remains in the root system, on residues, or in the soil where it can overwinter and survive as microconidia, macroconidia, and/or chlamydospores (**D**). Blue pigmented fungal masses can develop on infected roots (**D**).

CHAPTER 2

Coinfection of Soybean with *Fusarium virguliforme* and the Common Root Pathogens *F. acuminatum, F. oxysporum, F. solani*, and *Clonostachys rosea*

Abstract

Many diseases reduce soybean (*Glycine max*) yield every year, with root diseases often having the greatest impacts on yield. One important root pathogen of soybean is *Fusarium virguliforme* (*Fv*), the causal agent of sudden death syndrome (SDS). Although soybean roots from plants displaying SDS symptoms are commonly infected by multiple fungal pathogens, it is unknown how interactions between *Fv* and the other fungi may alter SDS disease development and soybean growth. In this study, we examined potential interactions between *Fv* and two isolates each of *F. acuminatum, F. oxysporum, F. solani,* and *Clonostachys rosea* in vitro and in soybean plants to evaluate their effect on SDS disease development and soybean growth. Results suggest that coinfection of soybean with Fv and *C. rosea* and *Fv* and *F. solani* can reduce SDS foliar disease severity (FDS) and that coinfection of soybean with *Fv* and *F. oxysporum* and *Fv* and *F. acuminatum* can increase FDS compared to the *Fv*-only treatment under growth chamber conditions. Coinfections of soybean with *Fv* and all eight test isolates individually did not increase or decrease levels of root rot or plant biomass compared to infection by *Fv* alone. These results corroborate in vitro interaction assays where the growth of *Fv* was not affected by the presence of the test isolates. This is the first study to investigate how *Fv* interacts with other common soybean root pathogens and how these interactions impact SDS disease development.

Introduction

Soybean (*Glycine max* (L.) Merr.) is a legume crop originating from East Asia and one of the most important crops grown worldwide[1]. In 2018, 123 million tons (4.54 billion bushels) of soybean were produced in the United States[2], with 10.6 million tons (389 million bushels) produced in Minnesota[3]. Although soybean is a well-suited for production in large areas of the United States, many diseases reduce soybean yield yearly, with root diseases having the greatest impacts on yield[5].

An important root pathogen of soybean is *Fusarium virguliforme* O'Donnell & T. Aoki (*Fv*), the causal agent of sudden death syndrome (SDS). In the United States and Ontario, Canada between 2010-2014 SDS caused the fourth largest reduction in soybean yields among all diseases, with losses estimated at 5.7 million tons (210 million bushels)[5]. Losses due to SDS result from damage to roots and leaves. *Fv* colonizes roots in early spring leading to severe root rot and crown necrosis[8]. Chlorosis and necrosis of leaf tissues, which typically occur later in the season during soybean reproductive stages, result from *Fv* toxins being translocated to leaves[30]. This can lead to premature defoliation and pod abortion[8]. Many factors contribute to SDS disease development including cultivar selection[32], *Fv* population levels in soil[165], soybean cyst nematode populations[35–37], production practices[38,39], and environmental conditions[7]. Soybean roots are commonly infected with multiple root pathogens when they are also infected with *Fv*[62,63], but it is unknown how other root pathogens may affect *Fv* infection, colonization, and foliar disease expression.

In a Minnesota survey identifying fungi associated with soybean roots in 2007 and 2008, the most commonly isolated genera were *Fusarium*, *Pythium, Phytophthora,*

Rhizoctonia, and *Clonostachys*[62]. Because of the frequency of their isolation and ability to infect soybean, understanding the interactions between *Fv* and these fungal genera would be valuable information for implementing disease management strategies. However, only one study to date has investigated interactions with *Fusarium virguliforme* specifically. Under greenhouse conditions, an experiment was conducted to determine if the presence of both *Fv* and *Fusarium graminearum* (*Fg*) resulted in an increased negative impact on soybean growth compared with each pathogen alone[64]. Interestingly, results showed that there was no evidence for increased plant damage from the *Fv* + *Fg* combined treatment compared with *Fv* and *Fg* alone[64]. A few studies other have reported interactions between other *Fusarium* spp. (not *Fv*) and *Pythium, Rhizoctonia,* and *Clonostachys*. For example, synergistic interactions between *Fusarium* and *Pythium* spp. on pea[65], snap bean[66], and peanut[67] were reported. A recent study, however, found no interactions between *Fusarium* and *Pythium* spp. on seedling disease in soybean[68]. Similarly, the interaction between *F. oxysporum* and *R. solani* in causing soybean root rot was shown to be additive[69], but no interaction was observed between *F. solani* f. sp. *phaseoli*, and *R. solani* on snap beans[66].

In contrast to the little information available on interactions between *Fusarium* spp. and *Pythium* and *Rhizoctonia*, there are many studies on *Clonostachys rosea* and *Fusarium* spp. interactions. *C. rosea,* a mycoparasite with a saprophytic life phase[70], has been of interest due to its potential as a biocontrol agent and its ability to parasitize some *Fusarium* spp[71,72]. In an in vitro study, DNA of *F. verticillioides* was reduced in the presence of *C. rosea,* suggesting that *C. rosea* actively destroyed the mycelium of *F. verticillioides*[73]. Additionally, *C. rosea* suppressed sporulation of *F. culmorum* and *F. graminearum* on wheat straw, and of *F. culmorum, F. graminearum, F. proliferatum* and *F. verticillioides*

on maize stalks[74]. More recently, *C. rosea* was reported to be pathogenic on soybean[75] and faba bean[76]. On soybean, *C. rosea* caused taproot necrosis and necrosis on leaf trifoliates and was shown to infect trifoliates systemically[75]. However, the impact of *C. rosea* infection on soybean production and how it interacts with *Fusarium* spp. in soybean roots was unknown.

In the Minnesota survey identifying fungal genera associated with soybean roots, the three most prevalent *Fusarium* spp. isolated were *F. oxysporum, F. solani,* and *F. acuminatum*[63]. These results are similar to those reported from a similar survey conducted in Iowa between 2007-2009[64]. Thus, based on the frequency of isolation, interactions between *Fv* and other *Fusarium* spp. may occur. Indeed, much attention has been given to the study of interactions among *Fusarium* spp. This is likely because many pathogenic *Fusarium* species can cause similar symptoms on the same host and form a disease complex[68,78]. Pathogens within a disease complex can overlap spatially and temporally on their host[69], and may affect each other positively (synergism), negatively (antagonism), or not at all (coexistence)[80]. Thus, there are many ways in which *Fusarium* species can interact while sharing the same host.

Most documented interactions between *Fusarium* species have been competitive[87–89] and result from one of three interactions: competitive exploitation, interference competition, or parasitic fitness[81–83]. Competitive exploitation refers to the ability of one fungus to obtain resources faster than another without direct interactions. For example, when grown together in vitro, the biomass of the maize pathogens *Ustilago maydis* and *Fusarium verticillioides* decreased due to the depletion of nutrient resources after an initial period of accelerated growth[84]. Interference competition involves a chemical or mechanical

barrier that limits a competing fungus from obtaining resources from a shared substrate. For instance, a *Phoma* sp. endophyte colonizing finger millet secretes tenuazonic acid that prevents growth of pathogenic *F. graminearum*[85]. Interference competition by means of a mechanical barrier was also demonstrated in finger millet where the bacterial species *Enterobacter* formed specialized root hairs that prevented entry of *F. graminearum*[86]. Lastly, parasitic fitness refers to the aggressiveness of an isolate. This is important for intraspecies specific competition and has been observed within *Fusarium* species[81,82]. When rye was coinoculated with four isolates of *F. culmorum*, disease severity was reduced compared to when rye was inoculated with only one isolate[81]. Competition between *Fusarium* spp. can also be host mediated, and infection by one species can activate host defenses against another. For example, infection of tomato by a nonpathogenic strain of *F. oxysporum* reduced colonization of roots by a pathogenic strain of *F. oxysporum*[89].

Interactions between *Fusarium* species can also be synergistic. For example, foot and crown rot of wheat are caused by a complex consisting of *F. graminearum, F. culmorum, F. poae,* and *F. sporotrichioides,* and the presence of *F. graminearum* increased the incidence of the other *Fusarium* species[90]. In a field study, maize ears inoculated with *F. graminearum* facilitated subsequent infections by *F. verticillioides*[91]. In addition, infection by one species can also suppress host defenses, making the host more susceptible to subsequent infections. This has been shown in maize where early infection by *F. verticillioides* suppressed the production of secondary defense metabolites; therefore, aiding in the success of colonization by several other *Fusarium* species[92].

Although soybean roots can be simultaneously attacked by multiple pathogens, little work has been done to investigate how pathogen coinfections may alter SDS disease

development. The goal of this work was to understand interactions between *Fv* and select fungal root pathogens of soybean in vitro and in planta. The primary objective of this study was to determine the effects of coinfection of soybean with *Fv* and *F. acuminatum, F. oxysporum, F. solani,* or *C. rosea* (pathogenic) on SDS development and soybean growth.

Materials and Methods

Isolate Selection

All fungal isolates used in this study were obtained from the lab of Dr. D. Malvick at the University of Minnesota. A single-spore isolate of *Fv,* Wa1-SS1, collected from a field in Waseca, MN [166] and eight test isolates of other species were used from a collection obtained from Minnesota soybean roots in 2007 and 2008. They included two single-spore isolates each of *F. acuminatum* (*Fa*)*, F. oxysporum* (*Fo*), *F. solani* (*Fs*)*, and Clonostachys rosea* (*Cr*) [62]. The test isolates selected were *Fa* 07-337 (Fa 337), *Fa* 07-353 (*Fa* 353), *Fo* 07-071 (*Fo* 71), *Fo* 07-321 (*Fo* 321), *Fs* 07-154 (*Fs* 154), *Fs* 08-064 (*Fs* 64), *Cr* NF-116 (*Cr* 116), and *Cr* NF-22 (*Cr* 22). The identity of the *Fv* isolate[166] and all test isolates[62] were confirmed morphologically and by partial sequencing of the translation elongation factor-1α. Further descriptions of the source and pathogenicity of isolates are provided in Table 2.1. Prior to these studies, all isolates were stored at 4°C in a soil culture from which subcultures were made and maintained on 0.5× potato dextrose agar (PDA) (Difco Laboratories, Inc., Detroit, MI) at 25°C in the dark for 2 weeks.

In vitro temperature growth studies

The radial growth rates of *Fv* and test isolates were examined at 15, 20, 25, and 30°C to determine the optimal growth rates for each isolate. Studies were initiated by transferring 6-mm plugs from the margin of actively growing, 2-week-old cultures to the center of Petri plates (9 cm) containing 1/2× PDA. Three replications were performed for each isolate at each temperature. After transfer, cultures were incubated in darkness at one of the four temperatures. Two measurements, perpendicular to one another, were taken of each colony diameter at days 4, 8, 11, and 15 and the average radial growth was calculated for each plate. The study was not repeated.

In vitro interaction assays

To characterize interactions between *Fv* and the test isolates in vitro, Petri plates (9 cm) containing 0.5× PDA were inoculated with *Fv* and one of the test isolates (dual treatments). For controls, *Fv* and test isolates were placed alone on the same medium 1 cm from the plate's edge. For dual treatments, *Fv* was transferred to plates 3 days before the test isolates were placed on the plates to account for its slow growth. *Fv* was placed 1 cm from the plate edge and test isolates were placed on the opposite side of the plate. For all isolates, 6-mm diameter plugs were taken from the actively growing margins of 2-week-old cultures. Plates were incubated in the dark at 25°C and assessed daily over a 2-week period. The radii of *Fv* colonies on the side adjacent to the test isolates were measured at 2, 4, 6, 8, and 11 days after test isolates were placed on plates. For control *Fv* cultures, the radius was measured in the direction of the colony closest to the plate's center. Radial measurements of test isolates on dual and control plates were taken in the same way. Dual plates were checked daily to record the duration until contact (days) between *Fv* and the

test isolates. The *Fv* control and dual treatments were replicated a total of eight times and test isolate controls were replicated four times in two replications of this study. The percent of inhibition of radial growth (PIRG) for *Fv* in the presence of each test isolate was calculated using the formula: (**A**– **B**) ÷ **A** × 100, where **A** = mean radial growth (mm) of *Fv* from control plates and **B** = mean radial growth (mm) of *Fv* from dual plates. PIRG was calculated for all test isolates in the presence of *Fv* in the same way.

Coinfection studies

Experiments were performed in a growth chamber to assess the effect of co-infestation with *Fv* and each test isolate on SDS disease development and seed yield. This experiment had 18 treatments: non-infested control, infestation with *Fv* alone (*Fv*-only), infestation with each test isolate alone (*Fa* 337-only, *Fa* 353-only, *Fo* 71-only, *Fo* 321-only, *Fs* 154-only, *Fs* 64-only, *Cr* 116-only, and *Cr* 22-only) and co-infestation with *Fv* and each test isolate (*Fa* 337+*Fv*, *Fa* 353+*Fv*, *Fo* 71+*Fv*, *Fo* 321+*Fv*, *Fs* 154+*Fv*, *Fs* 64+*Fv*, *Cr* 116+*Fv*, and *Cr* 22+*Fv*). One day prior to planting, inoculum for *Fv* and the test isolates was prepared from cultures grown on 0.5× PDA in darkness at 25°C for 4 weeks. Spore suspensions were prepared by adding sterile deionized water to each culture plate, the spores were dislodged with a sterile spreader, and spore suspensions were filtered through 3 layers of cheesecloth. Cultures were rinsed again with sterile water and the water containing spores was collected and filtered in the same manner. The spore density of each isolate suspension was estimated with a hemocytometer.

For each isolate included in a treatment, spores were mixed into the growth medium to obtain 2×10^3 spores/cm^3 of soil mix as follows. *Fv* and test isolate spore suspensions containing 5.6×10^6 spores were combined (or added alone for single infestation treatments)

and brought to a volume of 300 mL with sterile water. The diluted spore suspensions were added to 2600 mL of growth medium with 50 cm^3 of sterilized Bob's Red Mill coarse grind cornmeal (Milwaukie, OR). The soil-inoculum-cornmeal mixture was thoroughly mixed and divided among four 10.2-cm square pots. Five seeds of soybean cultivar MN1410 (susceptible to SDS and developed by the University of Minnesota) were placed in each pot and covered with a thin layer of soil, 2.5 cm of sand, and amended with 10 cm^3 of Osmocote 14-14-14 (Scotts Miracle-Gro Co., Marysville, OH). Pots were arranged in a randomized block design with four replications and incubated at 25°C with a 14-h photoperiod. Pots were watered daily to maintain adequate soil moisture for *Fv* infection and soybean growth. The experiment was replicated once.

Plants were visually assessed 28 days after planting for SDS foliar disease severity (FDS) on a scale of 1 -100 as the percentage of chlorotic and necrotic foliar tissue. Plants were carefully removed from pots, keeping root systems intact, and excess soil was removed. Root rot severity was visually rated as the percentage of the taproot that was rotted. In trial one, the fresh aboveground biomass of both plants was recorded for each pot. In trial two the fresh whole plant biomass (roots and shoots included) was recorded per pot. Disease data and biomass measurements were averaged for each pot.

To confirm that test isolates infected the roots, re-isolations were performed. Roots from each pot were surface sterilized in a 0.5% NaOCl solution for 3 min, rinsed in deionized water twice, and blotted dry. Roots were then cut into small sections and blended for 10 minutes using a Bullet Blender 5E with 6 stainless steel UFO beads (Next Advance, Troy, NY). Small samples were taken from the blended roots and embedded in 0.5× PDA media for isolation. Cultures were checked daily for mycelial growth of the respective

pathogen and cultures were transferred. Roots of plant from non-infested pots were also subjected to isolations, and neither *Fusarium* nor *C. rosea* were isolated from these plants. Isolates were identified morphologically based on colony characteristics and microscopic examination of macroconidia[93] and confirmed with DNA sequencing as follows. Genomic DNA was extracted from the mycelium using the FastDNA Kit (MP Biomedicals)[167]. Polymerase chain reaction (PCR) amplicons were generated using standard PCR (sPCR) in an Eppendorf Pro S Mastercycler (Eppendorf, Westbury, NY) using the primer pairs EF1/EF2[168] for *Fusarium* spp. and ITS1F/ITS4[169] for *Clonostachys rosea*. PCR amplification products were visualized via electrophoresis on a 1.5% agarose gel, and DNA was purified using an UltraClean PCR Clean-Up Kit (Mo Bio Laboratories, Carlsbad, CA) according to the manufacturer's instructions. Sequencing of the internal transcribed spacer (ITS) for *C. rosea* and the translation elongation factor-1α for *Fusarium* spp. was performed by the University of Minnesota BioMedical Genomics Center (St. Paul). Sequences were subjected to BLAST search against the NCBI GenBank database.

To confirm *Fv* infection, all treatments that were infested with *Fv* were also subjected to real-time PCR (qPCR) for the specific detection of *Fv*. DNA was extracted from 0.1 g of the blended root samples using a modified FastDNA® protocol[167], and qPCR was performed using a Bio-Rad CFX96 Real-Time System (software v. 4.1.2433.1219) with SsoAdvanced Universal Probes Supermix (Bio-rad Laboratories, Hurcules, CA). Each well contained a 25-µl reaction mixture including 12.5 µl of supermix, 1.125 µl of both primers FvIGS-F1/ FvIGS-R3[37], 2.5 µl of the probe FvIGS-Probe2[37], 2.75 µl of molecular grade water, and 5 µl of the DNA sample. Thermal cycling parameters consisted of 2 min at 50°C and 10 min at 95°C, followed by 40 cycles of 15 s at 95°C and 1 min at 66°C[37].

Statistical analysis.

For in vitro temperature assays, the radial growth at day 8 was used for all analyses. For each isolate, comparisons of radial growth between temperatures were made using analysis of variance (ANOVA) and means were separated using Tukey's HSD test. For each temperature treatment, an ANOVA was performed to compare the growth of test isolates. When significant differences were found, comparisons were made between the growth of test isolates and *Fv* using the Dunnett's t test.

For in vitro dual interaction assays the radial growth at day 8 was used for all calculations and analyses. After 8 days some isolates had grown to the edge of the plate and measurements could no longer be taken. The radial growth of *Fv* in the presence of the test isolates was analyzed using an ANOVA. ANOVA was also used to compare the growth of each test isolate on control plates to their growth in the presence of *Fv.*

For the growth chamber coinfection studies, trials were analyzed separately due to significant differences in root rot and foliar disease severity between trials. The root rot severity data was subjected to ANOVA and means were separated using the Tukey's HSD test. Only treatments with *Fv* were included in the analysis of foliar disease severity (FDS) data. FDS data was subjected to ANOVA and the Dunnett's t test was used to compare means to the *Fv* only treatment. Similarly, for biomass data, when differences among treatments were detected using ANOVA, all treatments were compared to the *Fv* only treatment using the Dunnett's t test. All statistical tests were performed in R version 3.6.1 and the significance level used was 0.05.

Results

In vitro temperature growth studies

All isolates except *Cr* 116 had optimal growth at 25°C, with some growing optimally at more than one temperature (Fig. 2.1). Significant differences in the growth of isolates at different temperatures were detected. The *F. acuminatum* isolates grew most at 20°C and 25°C and their growth was reduced significantly at 15°C and at 30°C. This trend was also apparent for the *Fv* isolate Wa1-SS1, with the most significant reduction in growth at 15°C. Both *F. oxysporum* isolates grew most at 25°C and 30°C, with reductions in growth seen at 15°C. Similar results were found for *F. solani* isolates. Isolate *Cr* 116 was especially sensitive to different temperatures; it grew best at 30°C and with each 5°C drop in temperature the isolate's growth was significantly reduced. In contrast, isolate *Cr* 22 grew similar at temperatures between 20°C and 30°C, but its growth was reduced at 15 °C. All test isolates grew significantly faster than *Fv* in all four temperature treatments.

In vitro interaction assays

Growth of *Fv* in vitro was not increased or decreased by any of the test isolates in the dual assays (Table 2.2). In contrast, the growth of *F. acuminatum* and *F. oxysporum* isolates was inhibited 16.9 % to 27.3%, respectively, in the presence of *Fv* with a visible narrow demarcation line between the test isolates and *Fv* (Table 2.3 and Fig. 2.2). The growth of *F. solani* and *C. rosea* was not increased or decreased significantly by *Fv* (Table 2.3), and although no obvious inhibition zone was observed the mycelium of *C. rosea* and *F. solani* within the interaction zones was visually less dense than in the rest of the colony.

Coinfection studies.

Each of the *F. solani* and *C. rosea* isolates incited root rot on soybean similar in appearance to symptoms caused by *Fv* (i.e., dark discoloration of roots) (Fig. 2.3). Roots from pots infested with *F. acuminatum* and *F. oxysporum* isolates had minor root discoloration that was similar in appearance to that observed on roots of the non-inoculated control plants (i.e., light brown discoloration only), and thus we considered these isolates non-pathogenic under the conditions used in this study (Fig. 2.3).

Significantly more root ($F_{1,6}$=9.8, P= 0.02) and foliar ($F_{1,6}$=15.75, P= 0.007) disease developed in the *Fv*-only treatment in trial two compared to trial one (Tables 2.4 and 2.5, and Figs 2.4 and 2.5). There were no significant differences in root rot severity between trials for single inoculation treatments ($F_{1,70}$=0.09, P= 0.76), but significantly more root rot ($F_{1,70}$=50.2, P= < 0.001) and foliar disease ($F_{1,70}$=52.53, P= < 0.001) developed in trial two compared to trial one for the *Fv* coinfected treatments.

No coinfection treatment significantly increased or decreased root rot severity compared to the *Fv* only treatment in either trial (Figs 2.4 & 2.5). In trial one, *Fa* 337-only, *Fa* 353-only, *Fo* 71-only, *Fo* 321-only, *Cr* 116-only, *Cr* 22-only, and the non-inoculated control had significantly lower levels of root rot compared to the *Fv*-only treatment. Similar results were seen in trial two, except the treatment *Fs* 64-only also had significantly less root rot compared to the *Fv*-only treatment. The level of root rot severity incited by isolates of *F. solani* differed in both trials. Significantly more root rot was caused by *Fs* 154-only compared to *Fs* 64-only (Figs 2.4 & 2.5). No differences in root rot severity between isolates of *F. acuminatum, F. oxysporum,* or *C. rosea* were seen in either trial.

No coinfection treatment significantly increased or decreased foliar disease severity (FDS) compared to the *Fv*-only treatment in trial one, however, FDS was unusually low in the Fv treatment too (Fig 2.6). In trial two, the coinfection treatments *Fo* 71 + *Fv* (P=0.08) and *Fa* 337 + *Fv* (P=0.096) had significantly higher FDS compared to the *Fv*-only treatment, and *Cr* 22 + *Fv* (P=0.045), *Cr* 116 + *Fv* (P=0.026), and *Fs* 154 + *Fv* (P=0.003) had significantly less (Fig 2.6).

No treatment differences in aboveground biomass were detected between treatments in trial one ($F_{17, 54}$=1.4, P= 0.19) (Table 2.4). In trial two, significant differences in whole-plant biomass were measured among treatments ($F_{1,69}$=7.4, P= <0.001) (Table 2.5). Treatments that had significantly higher whole-plant biomass compared to the *Fv*-only treatment were *Cr* 116-only (P=0.047), *Cr* 22-only (P=0.099), *Fa* 337-only (P=0.0015), *Fa* 353-only (P=0.021), *Fo* 321-only (P=0.069), *Fs* 154-only (P=0.007), and the non-infested control (P=0.016).

Re-isolation of test isolates from inoculated roots was successful except for *F. acuminatum* isolates in both trials. In trial one, the frequency that test isolates were successfully isolated and confirmed with sequencing from the treatments *Fo* 71-only, *Fo* 71 + *Fv, Fo* 321-only, *Fo* 321 + *Fv, Fs* 154-only, *Fs* 154 + *Fv, Fs* 64-only, *Fs* 64 + *Fv, Cr* 116-only, *Cr* 116 + *Fv, Cr* 22-only, and *Cr* 22+ *Fv,* were 50, 75, 75, 25, 100, 50, 50, 50, 50, 75, 25, and 25 %, respectively. From one replication of the *Fa* 337-only treatment, the isolate was successfully isolated, but was not confirmed with sequencing. In trial two, re-isolation of test isolates was not as successful with a 25 % recovery from the treatments *Fo* 71-only, *Fo* 71 + *Fv*, *Fs* 64 + *Fv,* and *Cr* 116 only.

Fv was detected with a specific qPCR assay in all replications of the *Fv*-only treatment as well as the *Fo* 71 + *Fv, Fo* 321 + *Fv,* and *Cr* 116 + *Fv* treatments in trial one. For the remaining treatments, the percent of replications where *Fv* was detected was 75 % for *Fa* 337 + *Fv* and *Fa* 353 + *Fv,* 50 % for *Fs* 154 + *Fv,* and 25 % for *Fs* 64 + *Fv* and *Cr* 22 + *Fv.* Samples from trial 2 were not tested.

Discussion

Soybean roots from plants with and without SDS symptoms in fields are commonly infected by multiple fungal pathogens, but it is unknown if interactions between *F. vriguliforme (Fv)* and the other fungi may influence SDS disease development and soybean growth. In this study, we examined interactions between *Fv* and isolates of *F. acuminatum, F. oxysporum, F. solani,* and *Clonostachys rosea* in vitro and in soybean plants to evaluate their effect on SDS disease development and soybean growth under growth chamber conditions. Results suggest that coinfection of soybean with *Fv* and *C. rosea* and with Fv and *F. solani* can reduce SDS foliar disease severity, and that coinfection of soybean with Fv and *F. oxysporum* and with *Fv* and *F. acuminatum* can increase FDS compared to the *Fv*-only treatment. Coinfections of soybean with *Fv* and the test isolates did not reduce or increase levels of root rot compared to infection by *Fv* alone.

We tested all fungal isolates for their ability to grow across a range of temperatures (15 to 30°C) to determine which temperature to use for subsequent experiments. This was done because the outcomes of competitive exclusion can vary at different temperatures. For example, *F. moniliforme* outcompetes *F. graminearum* when coinoculated on maize ears because of its ability to grow at a broader range of temperatures compared to the latter[82]. Based on our results, we choose to perform the dual plate assay and coinfection

study at 25°C, which was best suited for the growth of all isolates except for *C. rosea* isolate *Cr* 116 that grew best at 30°C. Additionally, all test isolates grew significantly faster than *Fv* at 25°C, suggesting that they may have a competitive growth advantage over *Fv* at this temperature.

We evaluated the interactions between *Fv* and the test isolates in vitro. In culture, the growth of *Fv* was not measurably affected by *F. acuminatum, F. oxysporum, F. solani,* or *C. rosea* isolates. However, *Fv* negatively affected the growth of the *F. acuminatum* and *F. oxysporum* isolates. In contrast, the growth of *F. solani* and *C. rosea* isolates was not affected by *Fv*. It is unlikely that interference competition (i.e. chemical inhibition) occurs between *Fv* and *F. solani* and *C. rosea* isolates, but it may play a role in the interactions between *Fv* and isolates of *F. acuminatum* and *F. oxysporum.* Additional studies are needed to examine how *Fv* inhbits the growth of *F. acuminatum* and *F. oxysporum*.

Results from our coinfection studies suggest that coinfection of soybean roots by *Fv* and the test isolates *Cr* 116, *Cr* 22, and *Fs* 154 can reduce SDS foliar disease (trial two) compared to *Fv* alone. In the temperature studies, the growth of *Fv* was significantly slower compared to *Fs* 154, *Cr* 116*,* and *Cr* 22 at 25°C. Thus, one possible reason for the reduction in SDS foliar disease symptoms could be that these isolates colonized soybean roots faster than *Fv* through competitive exploitation and either reduced *Fv* colonization and growth, toxin production, or toxin translocation. However, future work is needed to elucidate the mechanism behind these findings. Interestingly, some results also suggest that interactions could increase FDS. In trial two, the coinfection treatments *Fo* 71 + *Fv* and *Fa* 337 + *Fv* had significantly higher (α=0.10) FDS compared to the *Fv*-only treatment. This warrants further investigation.

On the other hand, coinfections of *Fv* and the test isolates did not reduce the level of root rot compared to the *Fv* only treatment. These results corroborate the in vitro interaction assay where the growth of *Fv* was not affected by the presence of the test isolates. Future work will need to address how other fungi affect *Fv* colonization of soybean roots with quantitative measurements.

SDS root and foliar disease development are influenced by the abiotic environment[7]. Therefore, important factors such as temperature[171], light[172], soil type, and study duration were kept constant between trials by performing our coinfection study under growth chamber conditions. In addition, infestation of *Fv* and test isolates was done by mixing spore suspensions of known concentrations into soil, keeping inoculation rates of all isolates consistent across all treatments and between trials. Most studies on soilborne *Fusarium* spp. use a grain substrate colonized by the pathogen as inoculum[166,173]; however, quantifying this type of inoculum is not possible and thus with spore suspensions we much more precisely quantified inoculum amounts. However, even by controlling many aspects of the abiotic and biotic environment, significantly more SDS disease (root and foliar) developed in trial two compared to trial one, suggesting that conditions of trial two were more conducive for infection and/or pathogenicity of *Fv*. The one factor we did not control quantitatively was soil moisture, which can influence SDS disease development[40,45,53,61]. Both trials were watered in the same manner by keeping soil moist and never allowing it to dry out; however, it is possible that differences in moisture levels could have occurred between studies and influenced SDS disease development.

The isolates *Fa* 337, *Fa* 353, and *Fo* 71 were previously reported as pathogenic on soybean[62], but they were not pathogenic under the conditions of this study. Differences in

methods could have accounted for the differences in pathogenicity. In the previous study, pathogen-colonized sorghum was used to infest soil and quantification of the inoculum was not performed[62]; thus, the inoculum dose could have been significantly higher than that used in our study. Based on previous work[58], we chose an *Fv* inoculum rate of 2×10^3 spores/g soil that has been reported to naturally occur in soybean fields. Thus, results from our study attempted to mimic what transpires in the field. Additionally, the sorghum itself provides additional nutrients for the fungi which could have increased pathogenicity of the isolates in the previous study[62]. In the greenhouse, plants grown without the addition of crop residues or cornmeal exhibited low to no SDS disease symptoms, even in the presence of high population levels of *Fv*[173]. Thus, the addition of organic substrates strongly affects SDS development, which could also be true for *F. acuminatum* and *F. oxysporum* isolates.

In trial one, we did not see significant differences between the aboveground biomass between treatments which could have been due to the lack of SDS foliar disease symptoms. In trial two, we recorded whole plant biomass in the attempt to capture both the aboveground and belowground growth of soybean and again found that no coinfection treatments significantly increased or decreased plant biomass compared to the *Fv*-only treatment. These results agree with the trend we have seen, that *Fv* growth and root infection was not significantly affected by the test isolates. However, our study was short-term and did not capture potential long-term effects on plant biomass that may occur when plants mature[174,] and this should be addressed in future work.

In summary, this study provides insights into how SDS disease development is impacted by other root pathogens of soybean. Evidence suggests that *Fv* infection and root rot symptom development is not affected by the presence of other common coinfecting root

fungi; however, SDS foliar disease expression can be inhibited or increased by some fungal species. This work suggests that we may need to rethink how we approach understanding the risk and management of SDS and consider how other soybean root pathogens and other fungi may affect the development of this disease.

Table 2.1. Source of *Fusarium virguliforme* (*Fv*), *F. acuminatum* (*Fa*), *F. oxysporum* (*Fo*), *F. solani* (*Fs*) and *Clonostachys rosea* (*Cr*) isolates included in this study and their pathogenicity on soybean.

Test isolate	**County of origin** [a]	**Year isolated**	**Pathogenicity on soybean** [b]
Fa 07-337	Clay	2007	+
Fa 07-353	Marshall	2007	+
Fo 07-071	Brown	2007	+
Fo 07-321	Redwood	2007	-
Fs 07-154	Brown	2007	+
Fs 08-064	Redwood	2008	+
Cr 116	Marshall	2007	na [c]
Cr 22	Brown	2007	+
Fv (Wa1-SS1)	Waseca	2006	+

[a] Minnesota county

[b] Causes root rot symptoms on soybean under greenhouse conditions[62,75,166]

[c] No data on pathogenicity

Table 2.2. Effect of *Fusarium acuminatum* (*Fa*), *F. oxysporum* (*Fo*), *F. solani* (*Fs*) and *Clonostachys rosea* (*Cr*) isolates on the radial growth of *F. virguliforme* (*Fv*) in dual cultures.[a]

Test isolate	**Radial growth of *Fv* (mm)[b]**	**Inhibition of *Fv* (%) [c]**	**Time until contact between *Fv* and test isolate (days)**
Fa 07-337	22.0 ± 1.9	-2.9 ± 9.0	9.9 ± 0.9
Fa 07-353	21.0 ± 1.2	1.8 ± 5.6	10.8 ± 1.3
Fo 07-071	21.3 ± 1.6	0.6 ± 7.4	9.5 ± 0.8
Fo 07-321	21.1 ± 2.2	1.2 ± 10.4	12.9 ± 0.6
Fs 07-154	20.5 ± 1.1	4.1 ± 5.0	11.8 ± 0.7
Fs 08-064	21.8 ± 1.9	-1.8 ± 8.9	12.1 ± 0.8
Cr 116	20.9 ± 2.0	2.3 ± 9.5	11.9 ± 0.9
Cr 22	20.8 ± 1.8	2.9 ± 8.6	12.4 ± 1.7
None (*Fv* control)	21.4 ± 2.2	---	---

[a] There were no significant differences (α=0.05) in the radial growth of *Fv* in the presence of any of the test isolates compared to the *Fv* control. Values represent the means (± SD) of 8 replications combined over two replicated experiments for each test isolate.

[b] Radial growth of *Fv* on the side adjacent to the test isolate measured after 8 days.

[c] Percent of inhibition of radial growth of *Fv* in the presence of each test isolate.

Table 2.3. Effect of *Fusarium virguliforme* (*Fv*) on the radial growth of *F. acuminatum* (*Fa*), *F. oxysporum* (*Fo*), *F. solani* (*Fs*) and *Clonostachys rosea* (*Cr*) isolates in dual cultures.

Test isolate	Radial growth on dual plates (mm) [a]	Radial growth on control plates (mm) [b]	Inhibition of test isolates (%) [c]
Fa 07-337	35.8 ± 2.0 *	43.0 ± 1.4	16.9 ± 4.6
Fa 07-353	35.6 ± 1.8 *	43.8 ± 1.3	18.6 ± 4.0
Fo 07-071	38.3 ± 1.7 *	46.0 ± 1.4	16.7 ± 3.6
Fo 07-321	27.8 ± 1.0 *	38.3 ± 2.1	27.3 ± 2.6
Fs 07-154	31.3 ± 1.6	32.5 ± 1.3	3.9 ± 4.9
Fs 08-064	24.5 ± 0.5	24.7 ± 0.5	1.0 ± 2.2
Cr 116	29.3 ± 2.2	30.8 ± 1.3	4.9 ± 7.1
Cr 22	24.4 ± 2.7	25.8 ± 1.0	5.3 ± 10.4

[a] Radial growth of test isolate colony on the side adjacent to *Fv* after 8 days. Values represent the means (± SD) of 8 replications combined over two replicated experiments for each test isolate. The growth of isolates marked with * were significantly ($\alpha = 0.05$) reduced in the presence of *Fv*.

[b] Radial growth of test isolate colony not in the presence of *Fv* after 8 days. Values represent the means (± SD) of 4 replications for each test isolate.

[c] Percent of inhibition of radial growth of test isolates in the presence of *Fv*.

Table 2.4. Results from trial one for root rot severity, foliar disease severity, and aboveground fresh biomass of soybean plants from pots infested with *Fusarium acuminatum* (*Fa*), *F. oxysporum* (*Fo*), *F. solani* (*Fs*), and *Clonostachys rosea* (*Cr*) isolates alone or with *F. virguliforme* (*Fv*) under growth chamber conditions.[a]

Treatment	Root rot severity [b]		Foliar disease severity [c]	Biomass (g)[d]
Non-infested	2 ± 2.2	f	0	12.8 ± 1.7
Fa 337	3 ± 2.5	f	0	14.5 ± 1.8
Fa 353	5 ± 4.7	f	0	14.0 ± 1.7
Fo 71	8 ± 4.9	ef	0	13.4 ± 1.6
Fo 321	16 ± 11	bcd	0	14.1 ± 1.4
Fs 154	97 ± 1.6	a	0	13.2 ± 2.6
Fs 64	52 ± 28.8	cd	0	13.8 ± 1.9
Cr 116	39 ± 13.6	de	0	11.1 ± 2.1
Cr 22	32 ± 16.6	def	0	13.2 ± 1.8
Fa 337 + *Fv*	93 ± 3.7	a	16 ± 13.9	10.9 ± 3.6
Fa 353 + *Fv*	80 ± 12.1	abc	23 ± 25.1	12.0 ± 2.3
Fo 71 + *Fv*	88 ± 10.8	ab	28 ± 10	8.7 ± 5.6
Fo 321 + *Fv*	57 ± 16.6	bcd	12 ± 6.8	13.3 ± 1.7
Fs 154 + *Fv*	97 ± 2.5	a	1 ± 1.7	11.9 ± 2.7
Fs 64 + *Fv*	60 ± 15.4	bcd	0 ± 0	11 ± 1.6
Cr 116 + *Fv*	81 ± 6.9	abc	5 ± 5.5	12.7 ± 2
Cr 22 + *Fv*	83 ± 9.7	abc	11 ± 13.6	12.2 ± 1.6
Fv - only	74 ± 16.2	abc	13 ± 12.1	11.2 ± 3.7

[a] Values represent the means ± SD of 4 replications.

[b] Root rot severity was scored on a scale of 0 (no disease) - 100 (taproot completely rotted). Treatments with the same letter are not significantly different (α=0.05).

[c] Foliar disease severity was scored on a scale of 1 – 100 based on percentage of chlorotic and necrotic leaf tissue. Coinfection treatments were not significantly different (α=0.05) from the *Fv*-only treatment.

[d] No significant differences were detected between treatments (α=0.05).

Table 2.5. Results from trial two for root rot severity, foliar disease severity, and whole plant fresh biomass of soybean plants from pots infested with *Fusarium acuminatum* (*Fa*), *F. oxysporum* (*Fo*), *F. solani* (*Fs*), and *Clonostachys rosea* (*Cr*) isolates alone or with *F. virguliforme* (*Fv*) under growth chamber conditions.[a]

Treatment	**Root rot severity[b]**	**Foliar disease severity[cd]**	**Biomass (g)[d]**
Non-infested	1 ± 1.7 d	0 **	15.5 ± 1.3 **
Fa 337	0 ± 0 d	0	16.9 ± 1.3 *
Fa 353	10 ± 7.6 d	0	15.4 ± 0.6 *
Fo 71	5 ± 2.1 d	0	13.9 ± 2.3
Fo 321	5 ± 4.1 d	0	14.5 ± 1.2 **
Fs 154	98 ± 2.5 a	0	16.0 ± 1.7 *
Fs 64	72 ± 24.4 b	0	12.7 ± 4.6
Cr 116	46 ± 15 c	0	14.8 ± 1.8 **
Cr 22	40 ± 10.6 c	0	14.3 ± 1.6 *
Fa 337 + *Fv*	100 ± 0.8 a	93 ± 13.2 *	5.7 ± 2.6
Fa 353 + *Fv*	98 ± 2.5 a	65 ± 29.1	8 ± 3.7
Fo 71 + *Fv*	100 ± 0 a	95 ± 2.5 *	5.9 ± 2.4
Fo 321 + *Fv*	100 ± 0.8 a	70 ± 29.9	9.8 ± 3.1
Fs 154 + *Fv*	100 ± 0 a	10 ± 4.4 **	11.2 ± 2
Fs 64 + *Fv*	100 ± 0.8 a	68± 19.9	10.3 ± 3.9
Cr 116 + *Fv*	99 ± 1.7 a	21 ± 17.1 **	10.9 ± 2.2
Cr 22 + *Fv*	95 ± 4. a	24 ± 11.4 **	12.6 ± 2.8
Fv - only	100 ± 0.8 a	61 ± 20.7	9.5 ± 2.1

[a] Values represent the means ± SD of 4 replications

[b] Root rot severity was scored on a scale of 0 (no disease) - 100 (root completely rotted). Treatments with the same letter are not significantly different (α=0.05).

[c] Foliar disease severity was scored on a scale of 1 – 100 based on percentage of chlorotic and necrotic leaf tissue.

[d] Treatments that are significantly different from the *Fv*-only treatment are indicated (α=0.10 * and α=0.05 **)

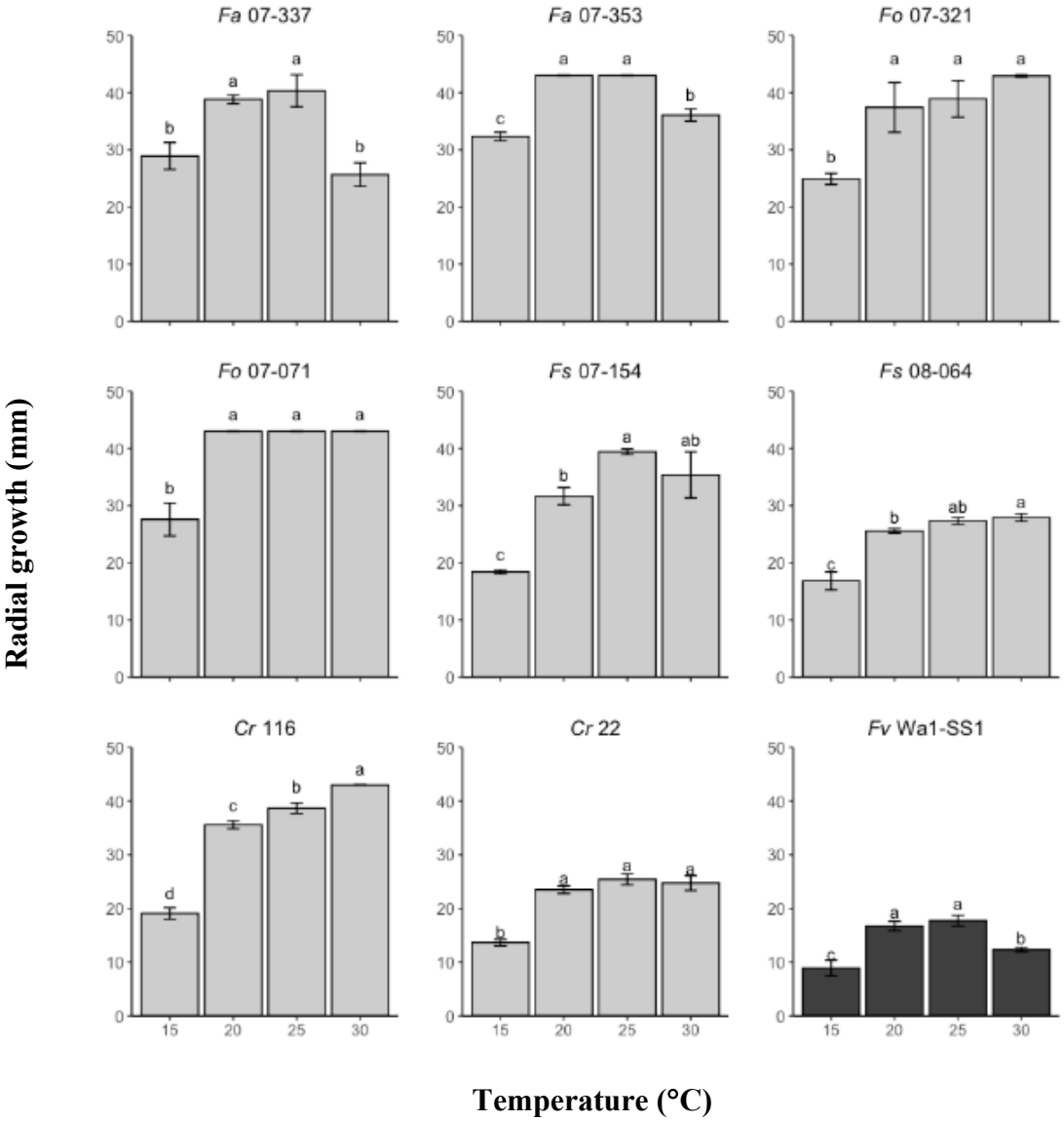

Figure 2.1. Radial growth of *Fusarium virguliforme* (*Fv*), *F. acuminatum* (*Fa), F. oxysporum* (*Fo*), *F. solani* (*Fs*) and *Clonostachys rosea* (Cr) isolates in culture at 15, 20, 25, and 30°C after 8 days on 0.5 × potato dextrose agar. Values represent the means (± SD) of three replications for each isolate at each temperature (*Fs* 07-154 replicated twice at 30°C). Means followed by the same letter are not significantly different across temperatures for each test isolate (α=0.05).

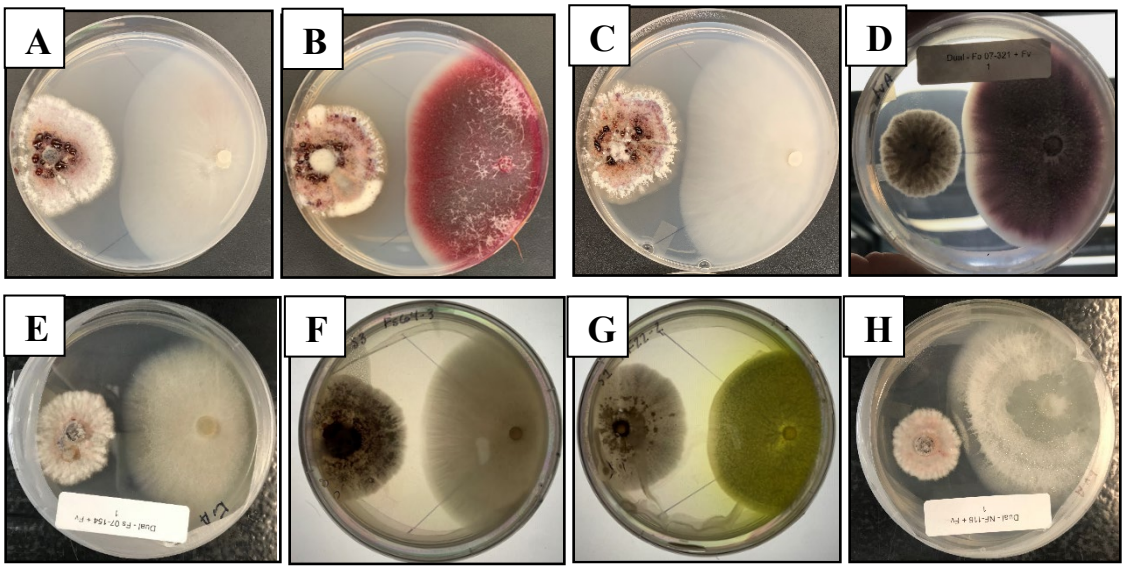

Figure 2.2. Growth of *Fusarium virguliforme* (*Fv*, left side in each photograph) in the presence of *F. acuminatum* (*Fa*), *F. oxysporum* (*Fo*), *F. solani* (*Fs*) and *Clonostachys rosea* (*Cr*) test isolates (right) in culture on 0.5× potato dextrose agar after 8 days of growth at 25°C. Test isolates included *Fa* 07-337 (**A**), *Fa* 07-353 (**B**), *Fo* 07-071(**C**), *Fo* 07-321(**D**), *Fs* 07-154 (**E**), *Fs* 08-064 (**F**), *Cr* 116 (**G**), and *Cr* 22 (**H**).

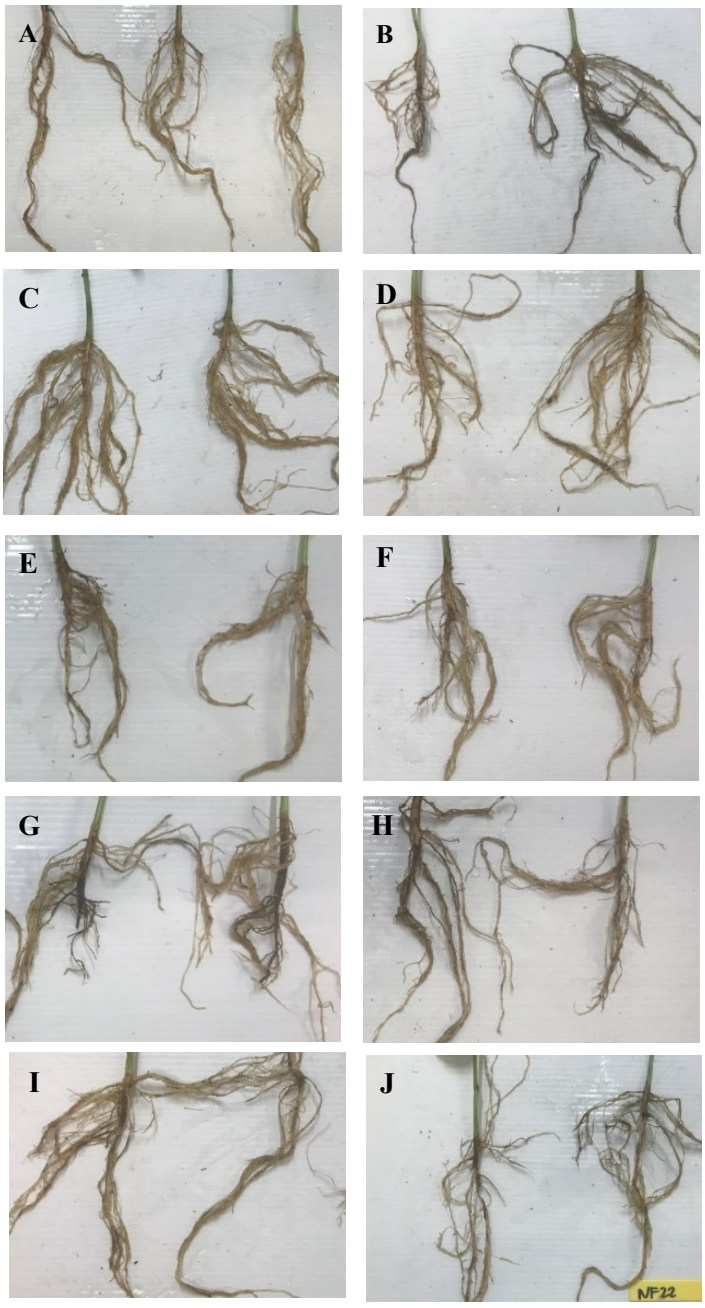

Figure 2.3. Soybean root rot symptoms on plants from pots infested with *Fusarium virguliforme* (*Fv*), *F. acuminatum* (*Fa*), *F. oxysporum* (*Fo*), *F. solani* (*Fs*) and *Clonostachys rosea* (*Cr*) isolates under growth chamber conditions. Non-infested control (**A**), *Fv* WaS-SS1 (**B**), *Fa* 07-337 (**C**), *Fa* 07-353 (**D**), *Fo* 07-071 (**E**), *Fo* 07-321 (**F**), *Fs* 07-154 (**G**), *Fs* 08-064 (**H**), *Cr* 116 (**I**), and *Cr* 22 (**J**).

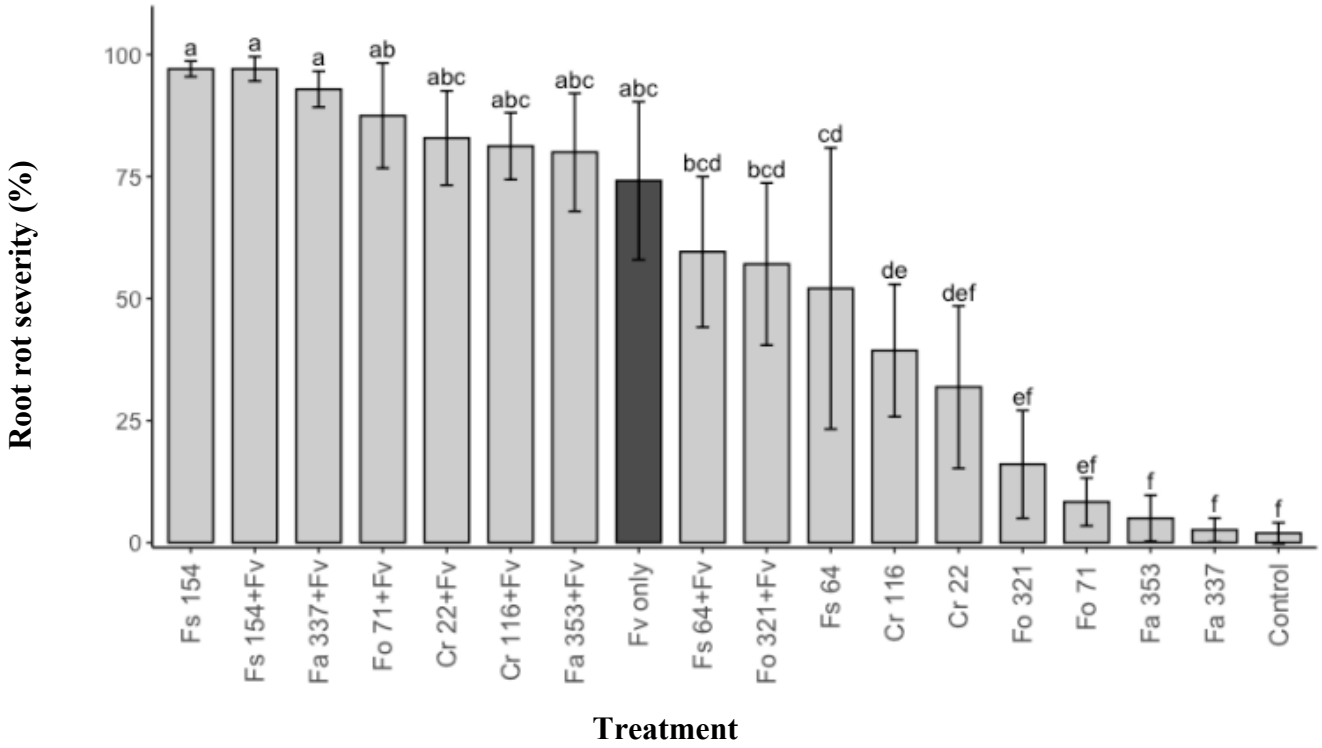

Figure 2.4. Root rot severity of soybean plants from pots infested with *F. acuminatum* (*Fa*), *F. oxysporum* (*Fo*), *F. solani* (*Fs*), and *Clonostachys rosea* (*Cr*) isolates either alone or with *Fusarium virguliforme* (*Fv*) under growth chamber conditions from trial one**.** Values represent the means ± SD of four replications. Root rot severity was scored on a scale of 0 (no disease) - 100 (root completely rotted) at the end of 4 weeks. Control pots were not infested. Treatments with the same letter are not significantly different (α=0.05).

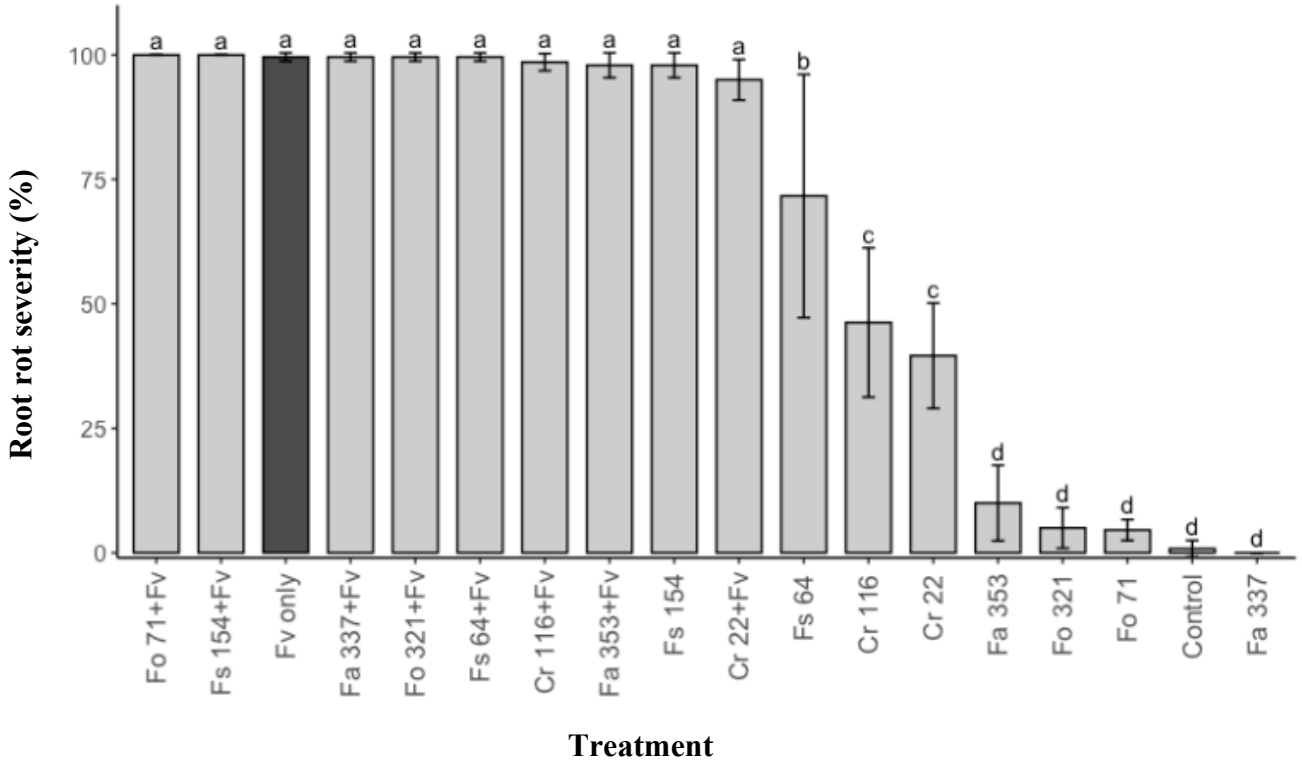

Figure 2.5. Root rot severity of soybean plants from pots infested with *F. acuminatum* (*Fa*), *F. oxysporum* (*Fo*), *F. solani* (*Fs*), and *Clonostachys rosea* (*Cr*) isolates either alone or with *Fusarium virguliforme* (*Fv*) under growth chamber conditions from trial two. Values represent the means ± SD of four replications. Root rot severity was scored on a scale of 0 (no disease) - 100 (root completely rotted) at the end of 4 weeks. Control pots were not infested. Treatments with the same letter are not significantly different (α=0.05).

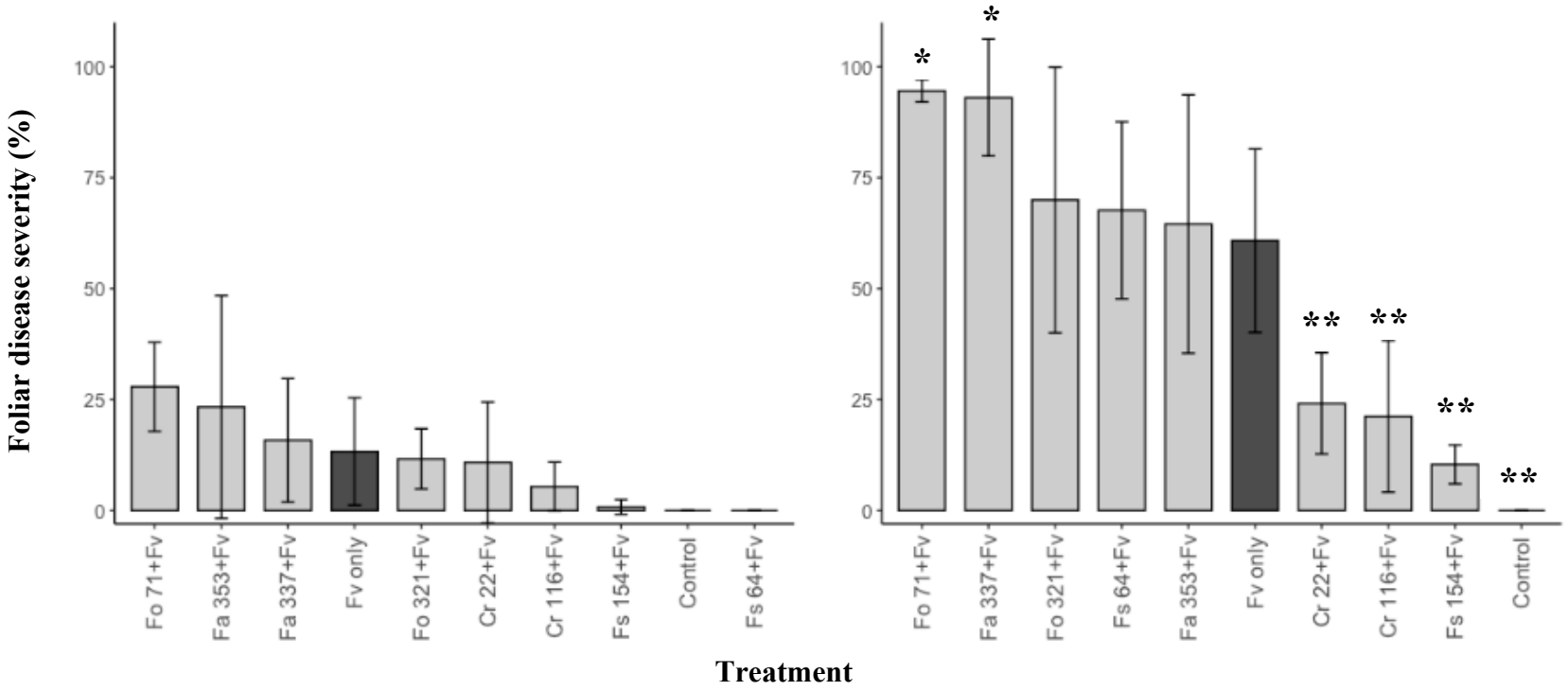

Figure 2.6. Foliar disease severity (FDS) of soybean plants from pots infested with *Fusarium virguliforme* (*Fv*) and one isolate of the species *F. acuminatum* (*Fa*), *F. oxysporum* (*Fo*), *F. solani* (*Fs*), and *Clonostachys rosea* (Cr) under growth chamber conditions from trial one (left) and trial two (right). Values represent the means ± SD of four replications. FDS was scored on a scale of 0 – 100 based on percentage of chlorotic and necrotic leaf area 4 weeks after planting and infestation. Control pots were not infested. Treatments that are significantly different from the *Fv*-only treatment are indicated (α=0.10 * and α=0.05 **). No differences in FDS values among treatments were detected in trial one.

CHAPTER 3

Interactions of a belowground fungal pathogen, *Fusarium virguliforme,* and an aboveground herbivore, *Aphis glycines*, on soybean

Abstract

Multiple biotic stressors including *Fusarium virguliforme* (*Fv*), the causal agent of soybean sudden death syndrome (SDS), and the soybean aphid (*Aphis glycines*) reduce soybean (*Glycine max*) yields annually in the United States. Although *Fv* and *A. glycines* occupy different plant compartments, with *Fv* infecting roots and secreting toxins into the xylem and aphids feeding on the phloem of leaves and shoots; they can occur on the same plants. In this study, we examined potential interactions between *Fv* and *A. glycines* in greenhouse, growth chamber, and field experiments to evaluate their effect on SDS disease development, aphid growth, and soybean growth. Overall, the results suggest that *Fv* and soybean aphids have minimal to no interaction with one another while co-occurring on soybean when SDS develops to low levels as was the case in these studies. There was no clear effect of soybean aphid herbivory on SDS foliar or root disease development in these studies nor did we detect a clear effect of combined soybean aphid herbivory and *Fv* infection on soybean growth. To our knowledge this is the first investigation into potential interactions between *Fv* and *Aphis glycines.*

Introduction

Soybean (*Glycine max* (L.) Merr.) is a legume crop originating from East Asia and is one of the most important crops grown worldwide[1]. Although soybean is well-suited for production in large areas of the United States, multiple biotic and abiotic stresses reduce production annually. Two important and widespread biotic stresses include *Fusarium virguliforme* O'Donnell & T. Aoki (*Fv*), the causal agent of sudden death syndrome (SDS) and the soybean aphid, *Aphis glycines* Matsumura (Hemiptera: Aphididae).

Sudden death syndrome was first detected in the U.S in Arkansas in the 1970's, and now occurs in most U.S. soybean production states and in Ontario, Canada[8–14,16–20]. Sudden death syndrome was estimated to cause the fourth largest reduction in soybean yield among all diseases in the United States and Ontario, Canada between 2010-2014 with total losses estimated at 5.7 million metric tons (210 million bushels)[5]. Yield losses resulting from infection by *Fv* range from slight to 100% on individual plants,[8,29] and result from two phases of SDS development in soybean. First, *Fv* infects roots and causes root rot and crown necrosis starting in early vegetative stages. Second, during soybean reproductive stages, *Fv* secretes fungal toxins into the xylem that lead to interveinal chlorosis and necrosis of leaves[30]. This can lead to premature defoliation and pod abortion[31]. Although *Fv* causes foliar symptoms, it only infects roots and lower stems.

Soybean aphid is an invasive pest in the U.S. from Asia. Since its first detection in the U.S in Wisconsin in 2000,[108] the soybean aphid has become the most important insect pest of soybean in the Midwest[109]. Injury to soybean by the soybean aphid includes wrinkled and distorted foliage, premature defoliation, stunting, and reductions in plant growth, pod size, and seed weight[115]. Soybean aphids also reduce photosynthetic rates[116],

vector plant viruses[117, 118], and secrete honeydew that stimulates sooty mold growth that can reduce photosynthesis[119]. Yield losses due to soybean aphid can reach 45% in soybean; however, the impact on yield is highly dependent on the plant developmental stage when initial infestation occurs[120,121].

Much research has focused on understanding the individual impacts of *Fv*[175,176] and soybean aphid[121,177,178] on soybean production, but information on interactions between the pathogen and insect and its consequence on plant health is lacking. Although *Fv* and soybean aphid occupy different plant compartments, with *Fv* in the roots secreting toxins into the xylem[179] and aphids feeding on the phloem of leaves and shoots[180]; interactions between these attackers may occur.

The vascular system of plants allows for long-distance communication between roots and shoots, enabling plants to mount whole-plant defense responses, regulate growth, and allocate resources[145]. The linking between roots and shoots allows for cross-compartment interactions between belowground and aboveground attackers through the activation of systemic induced defense responses and/or changes in host primary metabolism[141,146]. The activation of signaling pathways by pathogen or insect herbivore attack leads to systemic and whole-plant defense responses. These responses can directly affect the ability of the host to defend itself locally or "prime" distant plant parts for upcoming attacks[147]. Indeed, priming of defense responses can play an important role in mediating cross-compartment interactions between attackers. For instance, root herbivory by *Acalymma vitattum* on cucumber increased the host's defense to attack by the foliar oomycete pathogen downy mildew[148.] In pepper, aboveground herbivory by the aphid *Myzus persicae* primed systemic defense responses leading to reduced colonization of roots

by the bacterial pathogen *Ralstonia solanacearum*[149]. Whether or not aphids and *Fv* interact in this manner had not been previously studied. Secondly, herbivore and pathogen attack may also induce responses that alter the host's primary metabolism and may affect other attackers. We know that changes in amino acid concentrations of the phloem can have significant effects on aphid performance [160, 161, 162,181]. However, to date no studies have documented how *Fv* infection may affect the concentration of amino acids in phloem sap.

Many published studies indicate that cross-kingdom, plant-mediated interactions can be important, even when pathogens and herbivores are separated spatially[144]. However, the role these or other interactions may play in the coinfection of soybean by *Fv* and soybean aphid was unknown. In this study, greenhouse, growth chamber, and field experiments were conducted to address three objectives. 1) Determine if infection of soybean by *Fv* affects the preference and/or performance of subsequent colonization by soybean aphid, 2) Determine if soybean aphid populations on soybean affect the foliar or root disease severity caused by *Fv,* and 3) Determine if simultaneous infection of soybean by *Fv* and herbivory by soybean aphid influences seed yield. To our knowledge this is the first investigation into potential interactions between *Fv* and soybean aphid.

Materials and Methods

Fusarium virguliforme inoculum and Aphis glycines populations.

Single-spore isolates of *Fv,* Wa1-SS1, collected from a field in Waseca, MN[166] and Ne305, collected from a field in Iowa, were used in these studies. The identity of the Wa1-SS1 isolate was confirmed morphologically and by partial sequencing of the translation elongation factor-1α[167], and the isolate Ne305 was confirmed as *Fv* with SNP sequencing by GBS (Carried out by The Institute for Genomic Diversity, Cornell University)[182]. Prior

to these studies, isolates were stored at 4°C in soil cultures from which subcultures were made and maintained on 0.5x potato dextrose agar (Difco Laboratories, Inc.) at 25°C in darkness for 4 weeks prior to production of inoculum[183] for the studies. Inoculum was prepared by soaking sorghum seed soaked overnight, placing it into spawn bags with filter patches (Fungi Perfecti, Olympia, WA), and autoclaving twice. Agar pieces containing *Fv* were added to each bag, which was sealed, mixed, and incubated at 24°C for 2 weeks. After the sorghum was thoroughly colonized with *Fv*, it was dried. Non-inoculated sorghum was prepared for control treatments in the same manner. Soybean aphids used in these studies were taken from a laboratory colony maintained at the University of Minnesota-St. Paul[184].

Large-cage field study.

Large-cage field studies were conducted in 2017 and 2018 at the University of Minnesota Research and Outreach Center in Rosemount, MN and at the Iowa State University Northern Research Farm in Kanawha, IA. At each location, 24 plots were divided into four treatments in a completely randomized design. Each plot was 2.5-meters long, consisting of two rows (row spacing of 76.2-cm) seeded at 345,000 seeds/ha. Spacing between adjacent plots was 1.4 meters. The soybean varieties used in this study were susceptible to both the soybean aphid and *Fv.* The cultivar MN1410 (developed by the University of Minnesota) was used in MN and Syngenta S24-K2 (Syngenta AG, Basel, Switzerland) in IA. Plots were planted on 8 May 2017 and 10 May 2018 in MN and on 24 April 2017 and 18 May 2018 in IA. Treatments were (1) control plots with no aphids or *Fv*, (2) plots infested with aphids, (3) plots infested with *Fv*, and (4) plots infested with *Fv* and aphids. *Fv* treated plots (treatments 3 & 4) were infested at planting by adding 13 cc of *Fv*-infested sorghum seed/m of row in furrows. The same amount of sterile sorghum

was added to non-inoculated plots (treatments 1 & 2). A pre-emergent herbicide was applied to manage weeds, and hand weeding was performed to control weeds after growth stage VE. Starting ~ 2 weeks after planting in MN, plots were irrigated to receive at minimum 2.5 cm and 5.1 cm water per week in 2017 and 2018, respectively. Plots in IA received no supplemental irrigation.

Plots were inspected for soybean aphids when plants reached growth stage V3. If found, soybean aphids were removed by hand or with insecticide applications when necessary. At the MN location in both years, soybean aphids infested the plots prior to cages being placed and had to be removed. In 2017 aphids were removed from MN plots by applying λ-cyhalothrin (116 ml product per ha, Warrior II with Zeon Technology®, Syngenta, Greensboro, NC, USA) on 15 June, and a second application of a formulated mixture of λ-cyhalothrin and thiamethoxam (328 ml product per ha, Endigo ZC, Syngenta, Greensboro, NC, USA) was applied on 27 June. In 2018, MN plots were sprayed with a mixture of λ-cyhalothrin and thiamethoxam (328 ml product per ha, Endigo ZC, Syngenta, Greensboro, NC, USA) on 1 June to remove aphids. In both years at the IA location, aphids did not infest cages prior to the planned infestation date. Once plots were confirmed aphid-free, PVC frames (1.5×2.5 m) were placed over each plot and enclosed with NO-SEE-UM mesh cages (Quest Outfitters, Sarasota, USA) to prevent natural aphid colonization. Mesh cages were held down by large sandbags (Fig. 3.1).

Manual aphid infestation was performed at growth stage R3 at both locations. In 2017, plots (treatments 2 & 4) were infested with 200 mixed-stage aphids (i.e., nymphs + wingless adults) on 17 July at both locations. In 2018, 400 mixed aged aphids were added to each plot on 16 July in MN and 18 July in IA. Before infestation, plots were visually

divided into eight sampling sections, with four sections in each row (0.6 meter-length). In 2017, a leaf cutting with 25 mixed-stage soybean aphids was pinned to the lower surface of the uppermost fully expanded trifoliate of one plant per section (8 per plot). In 2018, each section received two leaf cuttings (16 per plot). All infestations were made using aphids taken from a laboratory colony except for in MN 2018. Many cages were moved due to strong winds during a storm on 17 June 2018 in MN and as a result, three plots were naturally infested with soybean aphids prior to the planned infestation date. One plot exceeded the economic threshold of 250 aphids per plant and was removed from the study. Aphids from this plot were used to infest the aphid treated plots in MN 2018.

Aphid populations were assessed weekly in MN in 2017 (6 June – 23 August), IA in 2017 (13 June – 23 August), MN in 2018 (25 June – 13 August) and IA in 2018 (5 July – 22 August). Mesh cages were removed briefly and replaced after aphids were counted from eight plants per plot (one randomly selected per section). In 2018, counts of natural enemies of aphids were recorded in the same manner.

Plots were visually assessed for SDS foliar disease incidence (FDI) and severity (FDS) for each plot section weekly after initial symptoms appeared. FDI was rated as a percentage of plants that had SDS foliar symptoms, and FDS was rated on a 1-9 scale based on the percentage of chlorotic and necrotic tissue[192]. Using these parameters, foliar disease index (FDX) was calculated using the formula: FDI × FDS ÷ 9[185] and FDX values were averaged for each plot. In 2017, the last sampling date was 37 days after initial aphid infestation in both locations. In 2018, the last sampling date was 35 and 38 days after initial aphid infestation for the MN and IA locations, respectively. On the last sampling date, aphid counts, foliar disease ratings and root ratings were determined. Two plants per plot

section (16 total/plot) were sampled and visually assessed for root rot severity. Severity was based on a scale from 0 - 100 based on the percentage of the taproot with SDS-like rot symptoms. Soybean aphid counts and disease ratings were averaged for each plot (i.e., experimental unit).

To confirm *Fv* infection, 50 % of the SDS plots were randomly selected and roots from these plots were subjected to real-time PCR (qPCR) for the specific detection of *Fv*. In addition, 6 % of plots non-infested with *Fv* were also tested to confirm that *Fv* infection did not occur. Roots were dried in a 95ºC chamber for 4 days and then ground with a Wiley Mill. DNA was extracted from 0.1g of the ground root samples using a modified FastDNA® protocol[167], and qPCR was performed using a Bio-Rad CFX96 Real-Time System (software v. 4.1.2433.1219) with SsoAdvanced Universal Probes Supermix (Bio-rad Laboratories, Hurcules, CA). Each well contained a 25-µl reaction mixture including 12.5 µl of supermix, 1.125 µl of both primers FvIGS-F1/ FvIGS-R3[37], 2.5 µl of the probe FvIGS-Probe2[37], 2.75 µl of molecular grade water, and 5 µl of the DNA sample. Thermal cycling parameters consisted of 2 min at 50°C and 10 min at 95°C, followed by 40 cycles of 15 s at 95°C and 1 min at 66°C[37]. Of the SDS plots subjected to qPCR, *Fv* was detected in 100% of the plots tested, indicating successful infection. *Fv* was not detected with qPCR in roots from non-infested SDS plots and no SDS foliar symptoms were seen in non-infested SDS plots.

Plots were also harvested for seed yield. The IA plots were harvested with a small plot harvester and yields were reported as kg/ha for both years. Due to severe lodging in MN during both years, 10 plants were randomly harvested by hand, seed was collected using a small hand thresher, and yields were determined as g seed/10 plants.

Small-cage field study.

A small field cage experiment was conducted at the University of Minnesota Research and Outreach Center in Rosemount, MN in 2017. The study was conducted as a randomized complete block design, with 10 replications (two per block) of four treatments. Treatments, field descriptions, and maintenance methods were the same as described for the large field cages in Rosemount, MN in 2017. The study was established on 24 April 2017 and 3 seeds were hand planted (7.6 cm apart from one another) in each plot. Plots were spaced 0.9 meters apart. The soybean variety MN1410 was used and *Fv* treated plots were infested at planting (4 g of *Fv*-infested sorghum per linear foot). On 15 June 2017 plots were thinned to one plant per plot, confirmed aphid-free, and caged with mesh over tomato cage frames. Soybean aphid plots were infested with 25 aphids per plant on 17 July 2017 using the same methods described for the large-cage study. Data collection and sampling occurred between 24 July and 15 August 2017 and followed the methods used in the large-cage study.

Greenhouse study.

The study was conducted as a randomized complete block design, with six replications of four treatments. Treatments were the same as those in the large-cage field study. *Fv* treatments were infested at planting by adding and thoroughly mixing 50 cc of *Fv* infested sorghum with soil in each 10.2-cm square pot. Sterile non-infested sorghum seed was added to control pots in the same manner. Each pot contained two plants. At the V1 growth stage, plants were infested with aphids by wedging a small piece of leaf containing 10 mixed-age aphids in the uppermost node of each plant. After infestation, mesh framed by wire tomato cages was placed over each pot (Fig. 3.2). Whole plant aphid

counts were determined weekly and FDI was visually assessed. At 18 days after aphid infestation, plants with intact roots were carefully removed from pots and soil was rinsed off. The fresh biomass of both plants (roots and shoots included) was recorded for each pot and root rot severity was visually rated as done in the field study. Soybean aphid counts, disease severity data, and fresh whole plant biomass were averaged for each pot. This study was repeated, however, there was a lack of *Fv* infection and aphid infestation, and no useful data was obtained from the second replication

Growth chamber study.

The study was conducted as a randomized complete block design, with 20 replications of two treatments. Treatments were *Fv* infested pots and control pots, all which were infested with aphids. The same *Fv* and aphid infestation methods were used as described for the greenhouse study. Ten *Fv* infested pots and 10 control pots, each with two plants per pot were randomized within each cage (i.e. block), which was enclosed with no-see-um mesh framed by PVC pipes (0.3 × 0.61 meters) (Fig. 3.3). Soybean aphid counts and root rot severity ratings were assessed as described for the greenhouse study. The final sample date was 18 days after aphid infestation. Data were averaged for each pot (i.e. experimental unit).

Statistical analysis.

In all studies, mean whole-plant aphid density was used to calculate cumulative aphid-days (CAD) which is an estimate of aphid abundance over time[186]. CAD data was transformed to log10 (x+1) to meet statistical assumptions in all studies except for the large-cage field study where no transformation was needed. Data from IA 2018 was not included in the analysis because SDS symptoms did not develop and thus interactions could

not be analyzed. Additionally, plots infested by natural aphid populations were excluded from analysis. Due to significant differences in variance for CAD between location-years, CAD data was analyzed separately for each location-year in the large-cage field study. Aphid growth rates were calculated separately for experimental units (i.e. plots in field studies and pots in the greenhouse study) by the slopes of the log-linear relationships of mean aphids per plant over time. Aphid growth rate was expressed as the mean log transformation of the number of aphids per plant per day. Treatments 2 (aphids only) and 4 (*Fv* + aphids) were compared when examining the effect of *Fv* on soybean aphid. Comparisons of CAD and population growth rates between *Fv*-infested and *Fv* control treatments were made by using analysis of variance (ANOVA). Location-year, block, and cage were also included as terms in the ANOVAs for the large-cage field, small-cage field, and growth chamber studies, respectively. For the large-cage field study, differences in aphid population growth rates between location-years were detected and means were separated with Tukey's HSD. Population growth rates were not analyzed in the growth chamber study.

Treatments 3 (*Fv* only) and 4 (*Fv* and aphid) were compared when examining the effect of soybean aphid on *Fv* and SDS. In the large-cage field study, FDX was transformed to log10 (x+1) values prior to analysis, with FDX as the response. Comparisons of SDS root rot severity and foliar ratings between aphid-infested and non-infested treatments were made using ANOVA. Location-year, block, and cage were included as terms in the ANOVAs for the large-cage field, small-cage field, and growth chamber studies, respectively. Due to the lack of foliar symptom development, aphid effects on SDS foliar disease severity could not be analyzed in the small-cage field and growth chamber studies.

For plant biomass and yield data from the greenhouse and large-cage field studies, respectively, comparisons between treatments were made using ANOVA and means were separated with Tukey's HSD. Further, yield data from MN 2017 and 2018 were combined for analysis and year and treatment were included as terms in the ANOVA. Due to differences in harvesting methods, yield data taken in IA 2017 were analyzed separately. All tests were performed in R version 3.6.1 and the significance level used was 0.05 for all statistical tests.

Results

Large-cage field study.

Aphid population growth differed among location-years but was not influenced by *Fv* and the low levels of SDS that developed in the large-cage field study (Table 3.1 and Fig. 3.4). Neither the *Fv* treatment ($F_{1, 22} = 0.21$, P= 0.65) or interactions between *Fv* treatment and location-year ($F_{2, 20} = 0.66$, P= 0.53) influenced aphid population growth rates. Similarly, no effect of *Fv* treatment on CAD was measured in IA 2017 ($F_{1,9}$=1.15, P=0.31), MN 2017 ($F_{1,7}$=0.32, P=0.59), or MN 2018 ($F_{1,4}$=0.24, P=0.65) (Fig. 3.4). However, aphid growth rates differed by location-year ($F_{2,22}$= 13.28, P= 0.0002). Growth rates in MN 2018 were significantly higher than they were in IA (P=0.0009) and MN (P=0.00012) in 2017. Growth rates were not significantly different between MN and IA in 2017 (P=0.48) (Fig. 3.4). Additionally, no statistical differences in predator populations on the last sampling date were detected between aphid and *Fv* + aphid treatments (Table 3.2).

SDS root rot severity differed among location-years, but not between treatments with and without aphids (Table 3.1 and Fig. 3.5). The average root rot severity across the three location-years in the plots without *Fv* was 5.6 ± 6.8 % (± SD). For all plots infested

with *Fv* in the three location-years where SDS developed, the average root rot severity was 58.3 ± 20.4 %. Root rot severity ratings were significantly higher in IA 2017 compared to MN 2017 (P=0.0012) and MN 2018 (P=0.00006). Root rot ratings did not differ significantly between 2017 and 2018 in MN (P=0.17). Aphid treatment ($F_{2,22}$=0.11, P=0.74) and the interaction between aphid treatment and location-year ($F_{2,20}$=0.098, P=0.91) had no effect on SDS root rot severity, but location-year alone was significant ($F_{2,22}$=15.18, P<0.05) (Fig. 3.5).

Aphid treatment and location-year had no clear effect on SDS foliar disease development (FDX). Neither aphid treatment ($F_{1,22}$ =0.47, P=0.50) nor the interaction between aphid treatment and location-year ($F_{2,20}$=0.10, P=0.90) was associated with the level of FDX (Table 3.1). However, FDX differed significantly by location-year ($F_{2,22}$=11.13, P=0.0005). FDX ratings were significantly lower in MN 2018 compared to IA 2017 (P=0.018) and MN 2017 (P=0.0002) but did not differ between IA 2017 and MN 2017 (P=0.164) (Fig. 3.5).

Soybean seed yield was not significantly influenced by SDS, aphids, or their interactions (Fig. 3.6). Yield was not influenced by these treatments ($F_{3,17}$ = 1.21, P=0.335) in IA 2017. Similarly, no effect of treatment ($F_{3,30}$ = 1.57, P= 0.22) was observed on yield in MN 2017 or 2018, but year had a significant effect on yield ($F_{1,30}$ = 7.81, P= 0.009). No interaction between treatment and year was detected ($F_{3,27}$ = 0.55, P= 0.65) for the MN yield data (Fig. 3.6).

Small-cage field study.

Fv treatment did not have an effect on CAD ($F_{1,\ 14}$=0.56, P=0.47) or on aphid population growth rates ($F_{1,\ 14}$=0.30, P=0.60) in the small-cage field study (Fig. 3.7).

Similarly, aphid treatment had no effect on SDS root rot severity ($F_{4,14}$=0.002, P=0.97) (Fig. 3.8). The *Fv* infested plots had root rot severity ratings (49.8% ± 29.8% (± SD)) that were significantly greater ($F_{1,78}$=114.4, P<0.001) than the non-infested plots (7.0% ± 6.4%). SDS foliar symptoms did not develop in the inoculated plots in the small-cages.

Greenhouse study.

The *Fv* treatment significantly reduced aphid population growth rate ($F_{1,10}$ = 9.44, P = 0.01), cumulative aphid days (CAD, $F_{1,10}$ = 7.66, P = 0.02), and total aphids plant^{-1} ($F_{1,10}$ = 10.8, P = 0.008) in the greenhouse study (Table 3.4 & Fig. 3.9). CAD was reduced 31.1%, growth rates were reduced by 11.7%, and total aphid count plant^{-1} at 18 DAI was reduced 42.9 % in the presence of *Fv.* Aphid treatment did not significantly influence SDS root rot severity ($F_{1,5}$ = 0.9, P = 0.4) or foliar disease incidence ($F_{1,5}$ = 0.51, P = 0.51) (Fig. 3.10). The *Fv* infested pots had root rot severity ratings of 69.6 % ± 24.3 % (± SD) that were significantly greater ($F_{1,22}$=82.3, P<0.001) than the non-infested plots (3.1 % ± 7.3 %). Plant biomass (g/plant) varied significantly by treatment ($F_{3,20}$= 6.69, P=0.003). The combined presence of *Fv* and aphids significantly reduced plant biomass by 37.4% compared to the control (P=0.007). The combined treatment also reduced biomass compared to plants infested only with aphids (P= 0.004) (Fig. 3.11).

Growth Chamber Study.

Fv treatment did not have an effect on cumulative aphid days (CAD, $F_{1,37}$ = 0.09, P = 0.77) in the growth chamber study (Fig. 3.12). No foliar SDS symptoms developed over the duration of the study, but root disease developed in the *Fv* infested treatments. Plants from the *Fv* infested pots had root rot severity ratings of 21.6 % ± 15.2 % (± SD) that were

significantly greater ($F_{1,38}$=19, P<0.001) than those from the non-infested pots (4.9 % ± 8.0%). SDS foliar disease symptoms did not develop in this study.

Discussion

Multiple biotic stressors including *F. virguliforme,* the causal agent of SDS, and the soybean aphid reduce soybean production annually in the United States. *F. virguliforme* and aphids often co-occur on soybean, thus interactions may occur between these pests. In these studies, we challenged soybean plants in controlled and field-based studies with *Fv* and aphids alone and combined, and then evaluated their effect on SDS disease development, aphid growth, and soybean growth. Although evidence was found that *Fv* infection may negatively affect aphid growth rate and CAD in greenhouse studies, similar results were not measured in field studies. Overall, the results suggest that *Fv* and soybean aphids have minimal to no interaction with one another in the field while co-occurring on soybean when SDS develops at low levels, as was the cased in most of the location-years in this study. We found no clear effect of soybean aphid herbivory on SDS foliar or root disease development in these studies, nor did we detect a clear effect of combined soybean aphid herbivory and *Fv* infection on soybean growth.

When examining how *Fv* and soybean aphids may interact while sharing a host, our focus was on broader impacts. We were interested in identifying how simultaneous *Fv* infection and soybean aphid colonization shapes the outcome of disease development, attacker success (i.e. aphid population growth), and soybean growth. We addressed the questions of whether development of SDS foliar symptoms hasten when plants are simultaneously infested with soybean aphids, and if prior infection with *Fv* reduces the

performance of soybean aphids? Answers to these questions could greatly change the way we manage these pests.

In the greenhouse study, aphid performance (growth rate & CAD) was reduced in pots infested with *Fv*. We know that early infection of roots by *Fv* leads to changes in gene expression and induction of ethylene and jasmonic acid production in soybean roots[187] , as well as elevated salicylic acid levels in leaf tissues affected by *Fv* toxins[188]. Previous studies have also shown that effective defense responses to soybean aphid are mediated by salicylic acid and jasmonic acid pathways in resistant plants[188,190]. Therefore, elevated levels of jasmonic acid due to *Fv* root infection and salicylic acid in leaf tissues due to toxin induced SDS symptoms may have caused the negative effects on soybean performance. However, before further studies are conducted to disentangle the possible roles of defense-related hormones on *Fv*-aphid interactions, this study should be repeated to confirm that *Fv* infection can consistently reduce aphid performance under controlled conditions.

In addition, our greenhouse study did not reflect the variation and complexity in the field, where *Fv* had no detectable influence on aphid performance. Uncontrolled environmental factors in the field could have masked potential negative effects of *Fv* infection on soybean aphid performance. One such important factor could be temperature which can influence soybean aphid growth[191]. We kept temperature constant in the greenhouse study but were unable to control temperature fluctuations in the field, which could have contributed to the different outcomes between the greenhouse and field studies.

In addition to changes in insect performance, root infections can alter the preference of aboveground insect attackers[192,193]. In fact, in a previous study the soybean cyst nematode nematode *Heterodera glycines* (SCN) was found to influence the behavior of

soybean aphid during its initital colonization of soybean, with alates prefering non-infected control plants compared to SCN infected plants[192]. Knowing this we designed our growth chamber study so that our results would capture the overall effect that *Fv* infection has on aphids including both changes to aphid preference and performance. We did this by allowing aphids to move freely between *Fv*-infested and non-infested pots. However, by taking this broader approach we could not conclude how *Fv* infection influenced aphid performance and preference during initital infestation. To do this a choice-test would need to be conducted where disease free and *Fv*-infected plants are presented to soybean aphids, and population counts would need to be taken hours after infestation (vs. ours where counts were taken days after)[192]. In conclusion, additional studies under more conditions are needed to examine whether *Fv* infection influences aphid preference.

No clear effect of aphids on SDS foliar or root symptom development was detected in any of our studies. We did not observe more or less foliar or root disease in the *Fv* + aphid treatment compared to plants challenged with *Fv* alone. Sudden death syndrome foliar disease symptoms did not consistently develop in these studies, which made it challenging to determine whether aphid herbivory affected foliar disease development. The inconsistency in SDS foliar disease development is not uncommon, partially because foliar symptoms are dramatically influenced by the abiotic environment[7]. In addition, aphid herbivory did influence SDS root rot in our studies. High levels of SDS root rot developed in the *Fv* infested treatments in all studies, indicating successful infection by *Fv.* Although, other pathogens can produce root rot symptoms, we inferred that the root rot was primarily due to *Fv* infection based on the much greater levels of root rot in infested treatments compared to controls.

The outcome of plant-mediated interactions between *Fv* and aphids may vary with the intensity of damage inflicted by attackers[144]. For example, the outcome of the interaction between soybean aphid and SCN is density dependent. It was found that nematode populations increase during low to moderate levels of aphid herbivory, and nematode populations decline when aphids heavily colonized plants[194,195]. This is possibly due to increased competition between the pests for limited resources[196]. Because interaction outcomes are likely to vary across severity gradients, our studies provide a partial picture of interactions between *Fv* and soybean aphid. In all studies, potential effects of severe levels of SDS foliar symptoms on aphid growth could not be evaluated due to the lack of severe foliar disease development. Low levels of foliar symptoms developed in the large-cage field study (Maximum FDX score < 50) and greenhouse study (maximum FDI rating < 50%), and no foliar symptoms developed in the individual field cage or growth chamber studies. In future investigations, *Fv*-aphid interactions should also be evaluated across different levels of SDS disease severity and aphid herbivory.

Although cross-compartment indirect interactions between these two organisms were the focus of this study, other more complex interactions may also be at work[141]. Infection by pathogens can modify the interaction between herbivores and their natural enemies, which has been reported previously, although not for cross-compartment interactions[156,163,164]. For example, *Brassica rapa* plants infected with powdery mildew produce significantly less volatiles in response to feeding by the herbivore *Pieris brassicae*. In response, *Cotesia glomerate*, a parasitic wasp of *P. brassicae,* was less attracted to powdery mildew-infected plants, suggesting that oviposition and feeding in mildew-

infected plants may be a survival strategy for *P. brassicae*[165]. It is not known how *Fv* may affect host volatiles, which may be an important area for future research.

We attempted to exclude natural enemies from our field studies with mesh cages, but they were not entirely effective. This was not unexpected because mesh cages often do not completely exclude all aphid natural enemies in natural ecosystems[197]. In MN 2018, low numbers of ladybeetles (Coccinellidae), minute pirate bugs (*Orius insidiosus*), and predatory flies (Syrphidae) were documented. Parasitoid wasps belonging to the Braconidae and Aphelinidae families were the most frequently observed natural enemies in the cages. However, in the location-year that parasitism was recorded for large-cages (MN 2018), only low levels of parasitism were observed compared to levels observed on other mesh caged studies[200]. Given the low numbers of natural enemies, we considered any predatory effects negligible on aphid populations. Although natural enemy counts were not recorded in 2017, the same natural enemies were observed as in 2018. Future studies may wish to address the effects of natural enemies on interactions between *Fv* and aphids.

In the greenhouse study, the combination of *Fv* and aphids reduced soybean plant biomass compared to control plants. The *Fv*-only treatment did not significantly reduce biomass compared to control plants and caused reductions similar to that measured in plants challenged with *Fv* + aphids. In contrast, the aphid-only treatment did not cause significant reductions in biomass compared to control plants and plant biomass was significantly higher compared to the combined *Fv* + aphid treatment. This suggests that *Fv* infection was likely the primary factor contributing to the reduction of biomass in the combined treatment. Although aphids alone did not reduce plant biomass in this study, they could affect soybean in other ways, e.g., pod size, seeds pod^{-1}, seed oil and protein

concentrations[121], not captured by our biomass measurement. In cases where soybean aphid populations are low and no visible injury symptoms are present, injury to soybean can occur via reductions in photosynthesis[116]. Also, our greenhouse study was short term and did not capture potential long-term effects of *Fv* infection and soybean aphid feeding on yield and plant biomass that may occur when plants reach maturity[120, 166, 174].

Sudden death syndrome and aphids can both significantly reduce soybean seed yield in the field[121,175–177]. However, our attempts to quantify the interaction of *Fv* infection and aphid herbivory on seed yield under field conditions in this study produced unclear results. Due to complications in harvesting techniques and data analysis, no effects of treatment on yield were detected in either location. In addition, the aphid populations and SDS foliar disease levels that developed in our study likely did not reach high enough levels to cause significant yield reductions[175,177] and thus effects on yield were likely too small to detect. Future work will need to address the combined effect of soybean aphid feeding and SDS disease on soybean production.

In summary, this study provides insights into interactions that may exist between *Fv* and soybean aphid. Our results suggest that *Fv* and soybean aphids have minimal to no interactions with one another while co-occurring on soybean under the conditions and disease levels evaluated in the field studies . However, multiple, complex interactions may shape the outcome of the *Fv*-aphid interactions investigated in this study. Interactions among different types of organisms that simultaneously infect the same and different organs on plants is an important and understudied topic for research that could lead to breakthroughs in understanding the ecology, production risks, and management of multiple stressors on crop productivity.

Table 3.1. Effect of *Fusarium virguliforme* (*Fv*) and *Aphis glycines* on aphid population growth and sudden death syndrome (SDS) root rot and foliar disease development in large microplot field studies. [a]

Location year [b]	Treatment [c]	*n*	Cumulative Aphid Days [e]	Total aphids [f]	Aphid growth rate [g]	Root rot (%) [h]	Foliar Disease Index [i]
IA 2017	*Fv* + aphid	5	1827.8 ± 644.2 [d]	214.5 ± 122.2	0.057 ± 0.01	77.5 ± 9.3	8.3 ± 3.8
	aphid	6	2246.6 ± 685.5	201.8 ± 94.0	0.052 ± 0.004	---	---
	Fv	4	---	---	---	78.5 ± 12.0	10.8 ± 11.1
MN 2017	*Fv* + aphid	5	2903.0 ± 1970.6	289.0 ± 216.2	0.054 ± 0.01	50.0 ± 20.9	22.0 ± 14.1
	aphid	4	1881.4 ± 866.3	133.9 ± 73.2	0.047 ± 0.01	---	---
	Fv	6	---	---	---	54.5 ± 16.33	20.0 ± 18.2
MN 2018	*Fv* + aphid	3	30248.3 ± 46290.7	2038.2 ± 2230.2	0.081 ± 0.02	40.3 ± 11.6	2.8 ± 3.5
	aphid	3	9585.9 ± 9733.2	1545.8 ± 1265.8	0.102 ± 0.03	---	---
	Fv	3	---	---	---	38.4 ± 10.0	0.8 ± 0.5

[a] No significant differences (α=0.05) in cumulative aphid days, total aphids, aphid growth rate, root rot severity or foliar disease index were detected between treatments, but there were significant differences between location-years (shown in Figures 3.4 and 3.5).
[b] MN = Rosemount, MN and IA = Kanawha, IA.
[c] Treatment: aphid=infested with *A. glycines; Fv* + aphids= infested with *Fv* and *A. glycines*; *Fv*=infested with *Fv;* (control not shown)
[d] Values represent the means ± SD.
[e] Plot averages of total aphid count plant^{-1} converted to CAD over the sample dates[186].
[f] Total aphid count plant^{-1} on the last sampling date
[g] Aphid growth rate estimated by the slopes of log-linear relationships of mean aphid abundance plant^{-1} within plots overtime.
[h] Rated on a scale from 0 (no root rot) to 100 (root completely rotted)
[i] Foliar disease index (FDX) of SDS was calculated using the formula FDX=FDI x FDS/9[185]. Disease incidence (FDI) was estimated as the percentage of plants in a plot with SDS foliar symptoms. Disease severity (FDS) was scored on a 0-9 scale based on the percentage of chlorotic and necrotic leaf tissue and premature defoliation. SDS foliar disease was not observed in Kanawha, IA 2018 (data not included in analysis).

Table 3.2. Natural enemies of the soybean aphid, *Aphis glycines,* counted at weekly intervals in large soybean microplot field studies infested with aphids or aphids and *Fusarium virguliforme* (*Fv*).[a]

Natural enemy [b]	**Treatment** [c]	**Day 0** [e]	**Day 7** [e]	**Day 14** [e]	**Day 23** [e]	**Day 28** [e]
Black mummies	aphid	0.0 [d]	0.0	0.0	0.7 ± 2.7	12.8 ± 15.8
	Fv + aphid	0.0	1.0 ± 3.6	0.3 ± 0.74	3.2 ± 6.2	9.0 ± 14.9
Brown mummies	aphid	0.0	0.0	0.00	0.2 ± 0.5	1.2 ± 2.7
	Fv + aphid	0.0	0.0	0.04 ± 0.20	0.8 ± 3.1	1.0 ± 3.8
Lady beetles	aphid	0.0	0.0	0.00	0.0	0.9 ± 2.6
	Fv + aphid	0.0	0.1 ± 0.5	0.08 ± 0.41	0.7 ± 1.8	0.5 ± 1.0
Minute pirate bugs	aphid	0.0 ± 0.2	0.0 ± 0.2	0.13 ± 0.34	0.2 ± 0.6	0.7 ± 0.9
	Fv + aphid	0.1 ± 0.3	0.0 ± 0.2	0.08 ± 0.28	0.1 ± 0.4	0.8 ± 1.1
Predatory flies	aphid	0.0	0.0	0.00	0.0 ± 0.2	0.0 ± 0.2
	Fv + aphid	0.0	0.0	0.00	0.1 ± 0.3	0.0

[a] Counts of natural enemies from cages in Rosemount, MN 2018. There were no significant differences (α=0.05) in predator counts between aphid and *Fv* + aphid treatments at 0, 7, 14, 23, and 28 days after *A. glycines* infestation.
[b] Natural enemy: Black mummies = aphids parasitized by Aphelinidae; brown mummies = aphids parasitized by Braconidae; lady beetles = Coccinellidae (adult, larvae, pupae), minute pirate bugs = *Orius insidiosus* (adult), predatory flies = Syrphidae (adult, larvae).
[c] Treatment: aphid = infested with *A. glycines; Fv* + aphid = infested with *Fv* and *A. glycines*; *Fv* = infested with *Fv* (data not shown) and unchallenged control (data not shown)
[d] Values represent the mean ± SD of counts plant^{-1}.
[e] Number of days after *A. glycines* infestation.

Table 3.3. Effect of *Fusarium virguliforme* (*Fv*) and *Aphis glycines* on aphid population growth and sudden death syndrome (SDS) development on soybean in the small-cage field study.[a]

Treatment [b]	***n***	**Cumulative Aphid Days** [d]	**Total aphids** [e]	**Aphid growth rate** [f]	**Root rot (%)** [g]
Fv + aphid	10	14,693 ± 10,978 [c]	1,922 ± 1,619	0.13 ± 0.02	50.0 ± 26.1
aphid	10	11,794 ± 8,032	1,295 ± 1,121	0.12 ± 0.03	5.5 ± 3.7
Fv	9	11.8 ± 33.3	1.3 ± 3.6	---	46.7 ± 35.4

[a] There were no significant differences (α=0.05) in aphid populations between aphid and *Fv* + aphid treatments or in SDS root rot severity between *Fv* and *Fv* + aphid treatments

[b] Treatment: aphid=infested with *A. glycines; Fv* + aphids=infested with *Fv* and *A. glycines*; *Fv*=infested with *Fv;* (control not shown).

[c]Values represent the means ± SD.

[d] Plot averages of total aphid count plant^{-1} converted to CAD over the sample dates[186].

[e] Total aphids plant^{-1} on the last sampling day.

[f] Aphid growth rate estimated by the slopes of log-linear relationships of mean aphid abundance plant^{-1} within plots overtime.

[g] Rated on a scale from 0 (no root rot) to 100 (root completely rotted)

Table 3.4. Effect of *Fusarium virguliforme* (*Fv*) and *Aphis glycines* on aphid population growth and sudden death syndrome (SDS) development on soybean under greenhouse conditions.

Treatment [t]	***n***	**Cumulative Aphid Days** [v]	**Total aphids** [w]	**Growth rate** [x]	**Root rot (%)** [y]	**Foliar disease severity** [z]
Fv + aphid	6	2410 ± 756 a[u]	373± 87 a	0.09 ± 0.01 a	73.8 ± 28.3 a	17.9 ± 12.5 a
aphid	6	3498 ± 579 b	653 ± 190 b	0.10 ± 0.01 b	3.3 ± 8.2	---
Fv	6	---	---	---	65.4 ± 21.4 a	24.6 ± 19.6 a
Control	6	---	---	---	3.0 ± 7.1	---

[t] Treatment: aphid = infested with *A. glycines; Fv* + aphid = infested with *Fv* and *A. glycines*; *Fv* = infested with *Fv*; Control = non-infested.

[u] Means (± SD) in the same column marked with the same letter are not significantly different (α=0.05).

[v] Total aphid count plant^{-1} converted to CAD over the sample dates[186].

[w] Total aphid count plant^{-1} 18 days after infestation.

[x] Aphid growth rate estimated by the slopes of log-linear relationships of mean aphid abundance plant^{-1} within pots overtime.

[z] Percentage of leaf area with SDS-like foliar symptoms.

Table 3.5. Effect of *Fusarium virguliforme* (*Fv*) and *Aphis glycines* on aphid population growth and sudden death syndrome (SDS) root rot severity on soybean under growth chamber conditions. [a]

Treatment [b]	***n***	**Cumulative Aphid Days** [d]	**Total aphids** [e]	**Root rot (%)** [f]
Fv + aphid	20	3098 ± 722 [c]	380 ± 138	21.6 ± 15.2
aphid	20	3303 ± 1936	493 ± 374	4.9 ± 8.0

[a] There were no significant differences (α=0.05) in CAD, total aphids, or root rot severity between treatments.

[b] Treatment: aphid = infested with *A. glycines; Fv* + aphid = infested with *Fv* and *A. glycines*; *Fv* = infested with *Fv* (not shown); control = non-infested (not shown).

[c] Values represent the means ± SD

[d] Total aphid count plant^{-1} converted to CAD over the sample dates[186].

[d] Total aphid count plant^{-1} 18 days after infestation.

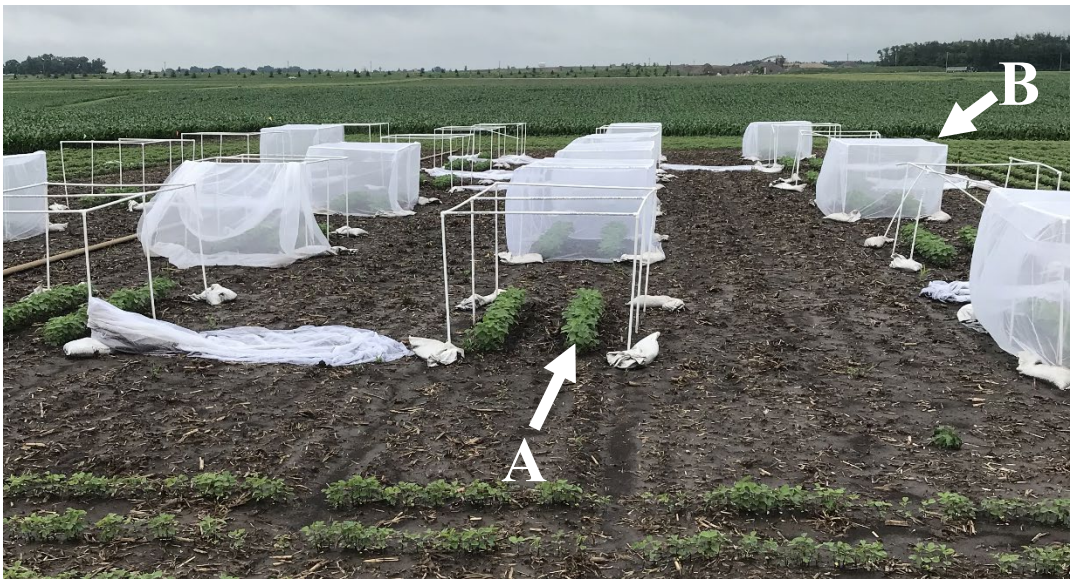

Figure 3.1. Caged microplots used to study interactions between *Fusarium virguliforme* and *Aphis glycines* on soybean in field studies. Plots were uncaged weekly to determine aphid counts (**A**) and then cages were placed over plots and held down with sandbags (**B**).

Figure 3.2. Individually caged pots used to study interactions between *Fusarium virguliforme* and *Aphis glycines* on soybean in a greenhouse.

Figure 3.3. Cages used to study interactions between *Fusarium virguliforme* and *Aphis glycines* on soybean in a growth chamber.

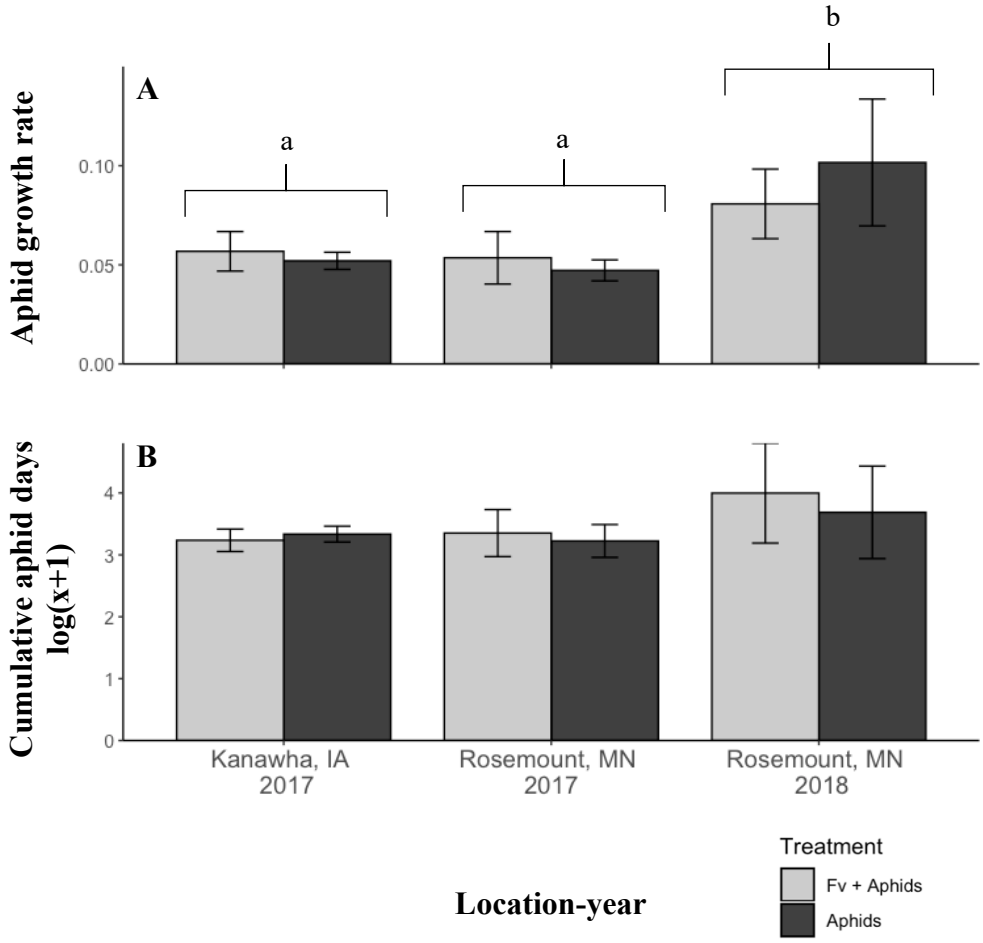

Figure 3.4. Effect of *Fusarium virguliforme* (*Fv*) on *Aphis glycines* population growth on soybean in large-cage field studies. The means ± SD of aphid growth rate plant^{-1} (**A**) and log transformation (x+1) of cumulative aphid days (CAD) plant^{-1} (**B)** are shown. In all 3 location-years, there were no significant differences (α=0.05) in aphid growth rates or CAD between aphids and *Fv* + aphid treatments, but differences in aphid growth rates were seen between location-years. In panel **A**, location-years with the same letter are not significantly different (α=0.05). There were no differences in CAD between location-years.

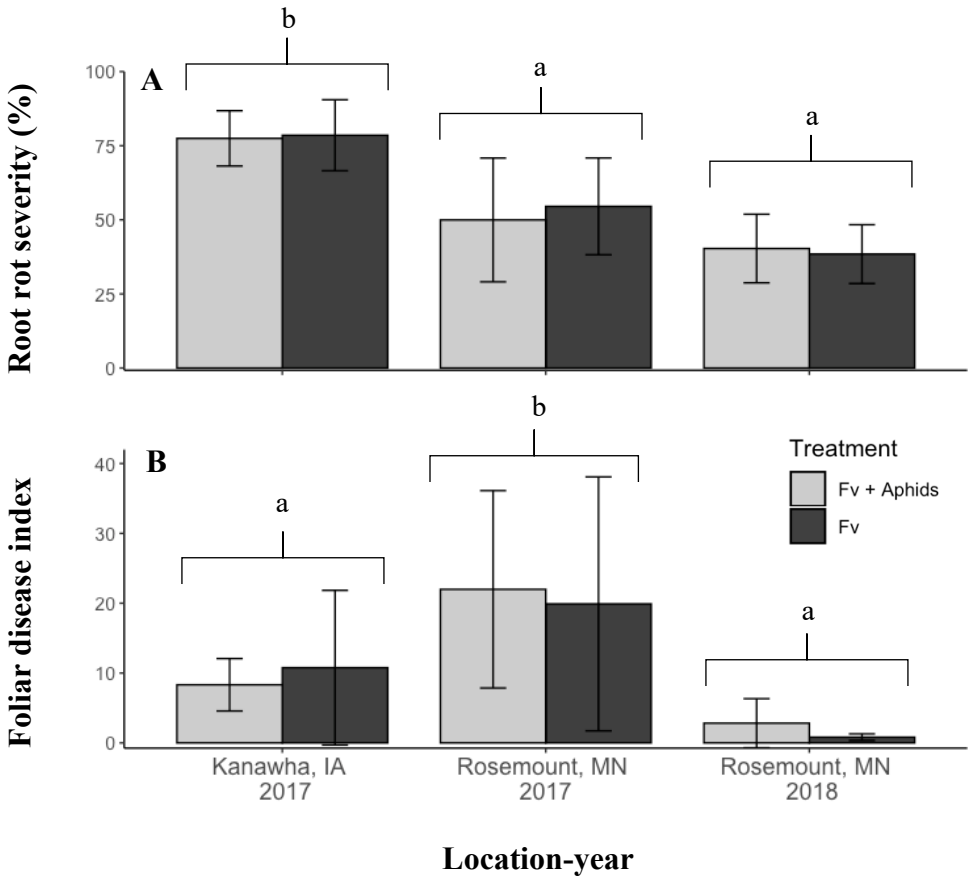

Figure 3.5. Effect of Aphis glycines on soybean sudden death syndrome (SDS) root rot and foliar disease severity in large cage field studies. The means ± SD of root rot severity per plant (**A**) and foliar disease index (FDX) per plot (**B)** are represented. In all three location years, there were no significant differences (α=0.05) in root rot severity or FDX between *Fv* and *Fv* + aphid treatments. Within panels **A** and **B**, location-years with different letters are significantly different (α=0.05).

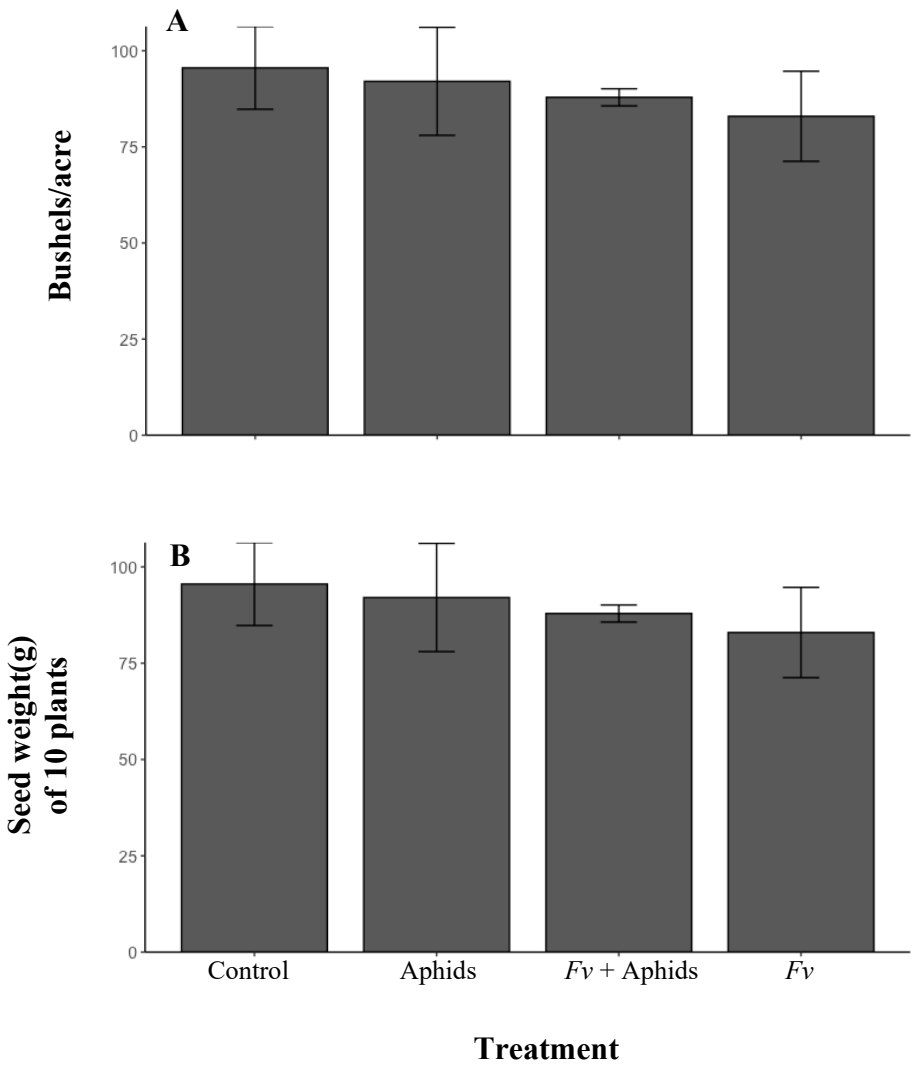

Figure 3.6. Soybean seed yield in large-cage field studies infested with different combinations of *Fusarium virguliforme* (*Fv*) and *Aphis glycines* (aphids). The means ± SD of bushels/acre from Kanawha, IA 2017 (**A**) and seed weight (g) ten plants^{-1} from Rosemount, MN (2017 & 2018) (**B**) are shown. There were no significant differences (α=0.05) in yield between treatments in Kanawha, IA 2017 or Rosemount, MN 2017 & 2018.

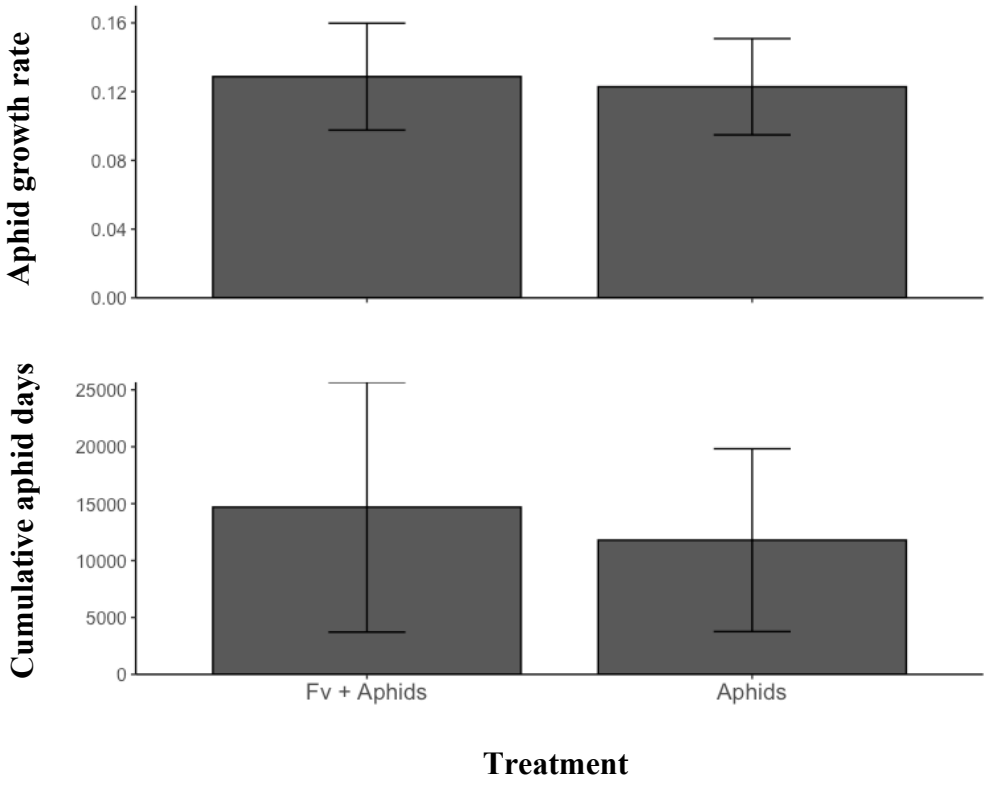

Figure 3.7. Effect of *Fusarium virguliforme* (*Fv*) on aphid population growth on soybean in a small-cage field study. The means ± SD of aphid growth rate per plant (**A**) and cumulative aphid days per plant (**B**) are represented. There were no significant differences (α=0.05) in aphid growth rates or CAD between aphid and *Fv* + aphid treatments.

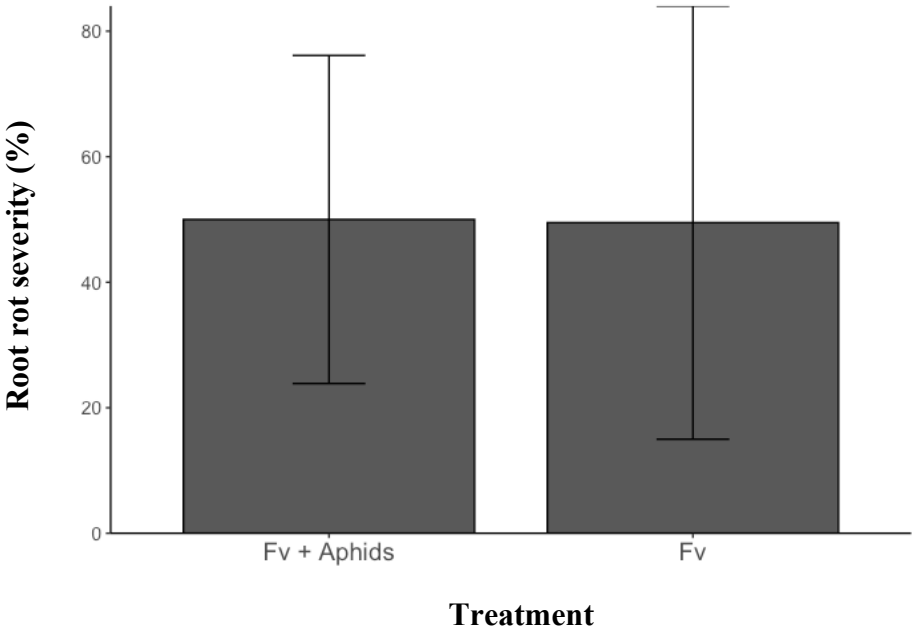

Figure 3.8. Effect of treatment with *Aphis glycines* on soybean sudden death syndrome (SDS) root rot severity in a small-cage field study. Values represent the means ± SD. There was no significant (α=0.05) differences in root rot between *Fv* and *Fv* + aphid treatments.

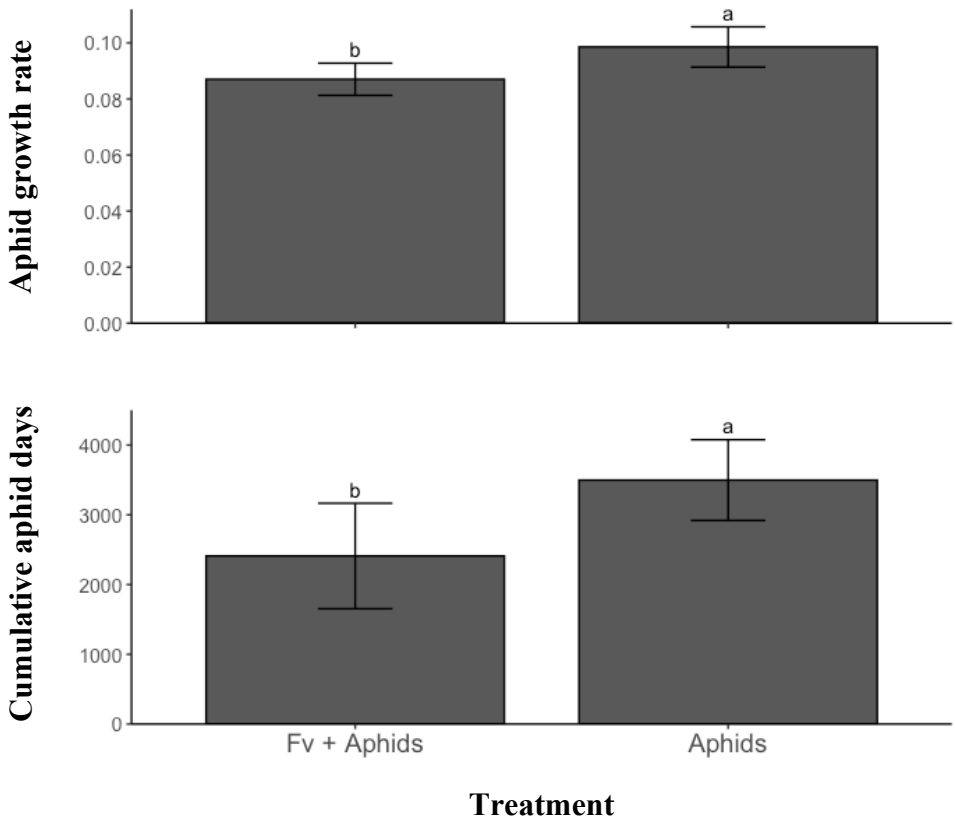

Figure 3.9. Effect of treatment with *Fusarium virguliforme* (*Fv*) on aphid population growth on soybean under greenhouse conditions**.** Values represent the means ± SD of aphid growth rate per plant (**A**) and cumulative aphid days per plant (**B)**. Aphid growth rates and CAD were significantly different between aphid and *Fv* + aphid treatments (α=0.05).

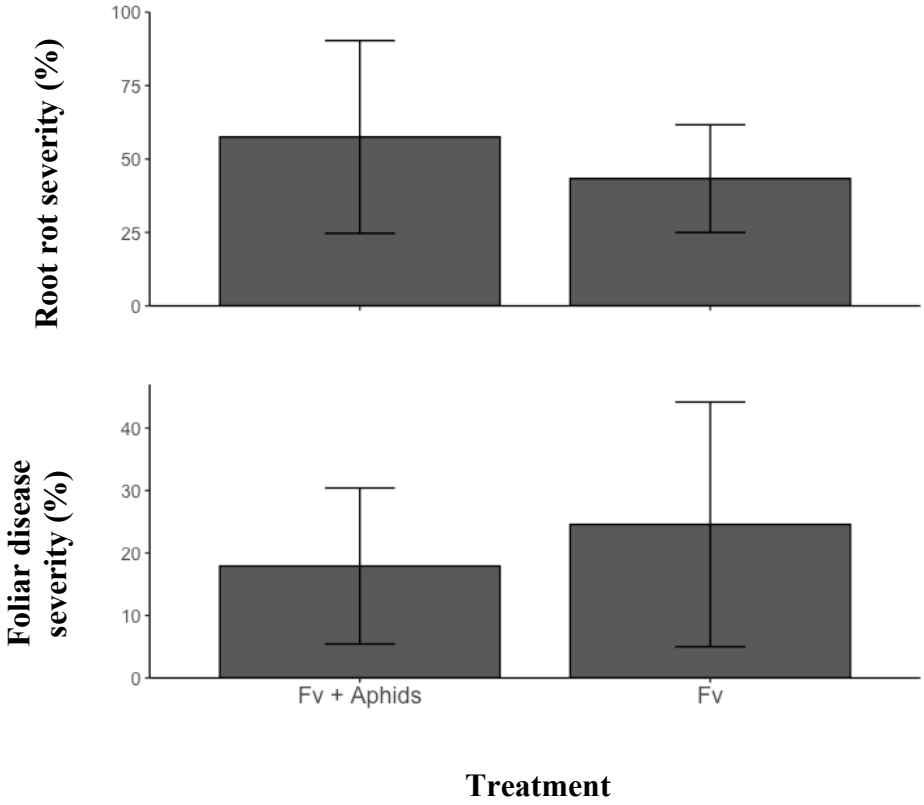

Figure 3.10. Effect of *Aphis glycines* on soybean sudden death syndrome (SDS) root and foliar disease development under greenhouse conditions. The means ± SD of root rot severity per plant (A) and foliar disease severity (FDS) per plant (B) are shown. There were no significant (α=0.05) differences in root rot severity or foliar disease severity between *Fv* and *Fv* + aphid treatments.

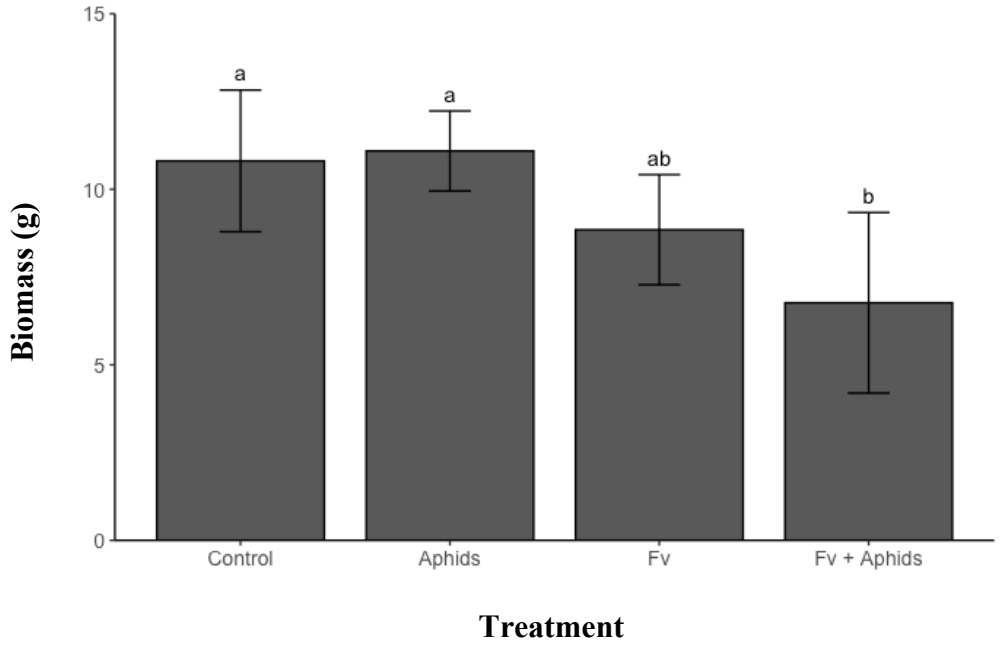

Figure 3.11. Effect of *Fusarium virguliforme* (*Fv*) and *Aphis glycines* on soybean biomass under greenhouse conditions. Values represent means ± SD of fresh biomass (g) per plant (roots and shoots). Treatments with the same letter are not significantly different (α=0.05).

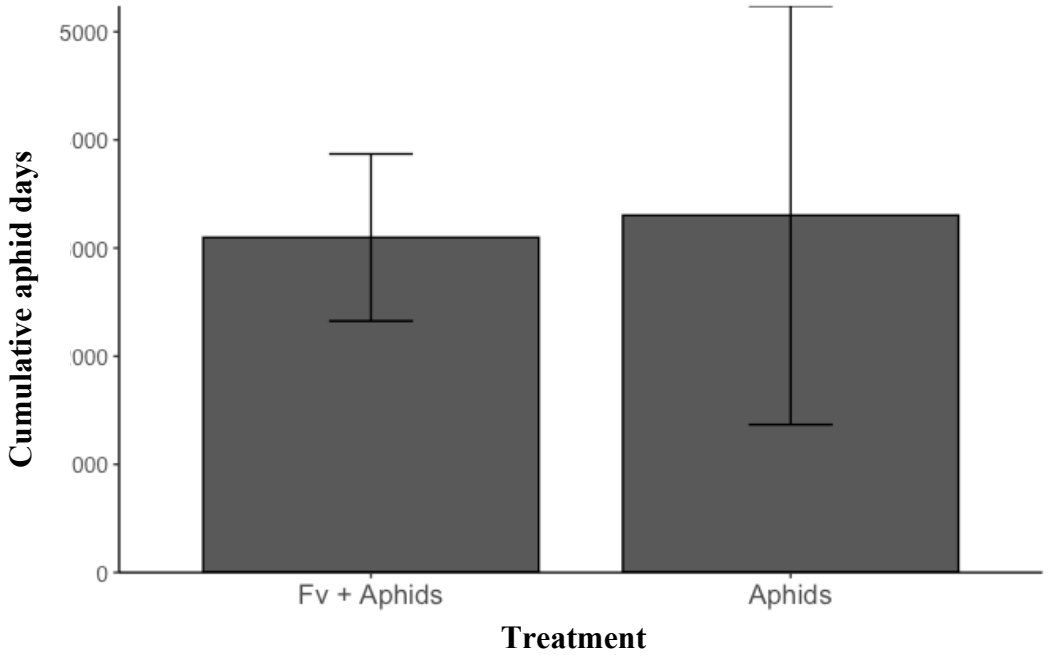

Figure 3.12. Effect of treatment with *Fusarium virguliforme* (*Fv*) on aphid population growth on soybean under growth chamber conditions. Values represent means ± SD of cumulative aphid days per plant. There were no significant (α=0.05) differences in cumulative aphid days between aphid-only and *Fv* + aphid treatments.

BIBLIOGRAPHY

1. Qiu, L. J. and Chang, R. Z. 2010. The Origin and History of Soybean. Page 1-23 in: The Soybean: Botany, Production and Uses.

2. USDA-NASS. 2019. Crop Production 2018 Summary. Report ISSN: 1057-7823. Page 3.

3. USDA-NASS. 2019. Minnesota Ag News – 2018 Crop Production.

4. USDA. 2015. USDA Coexistence Fact Sheets Soybeans.

5. Allen, T. W., Bradley, C. A., Sisson, A. J., Byamukama, E., Chilvers, M. I., Coker, C. M., Collins, A. A., Damicone, J. P., Dorrance, A. E., Dufault, N. S., Esker, P. D., Faske, T. R., Giesler, L. J., Grybauskas, A. P., Hershman, D. E., Hollier, C. A., Isakeit, T., Jardine, D. J., Kelly, H. M., Kemerait, R. C., Kleczewski, N. M., Koenning, S. R., Kurle, J. E., Malvick, D. K., Markell, S., G., Mehl, H. L., Mueller, D. S., Mueller, J. D., Mulrooney, R. P., Nelson, B. D., Newman, M. A., Osborne, L., Overstreet, C., Padgett, G. B., Phipps, P. M., Price, P. P., Sikora, E. J., Smith, D. L., Spurlock, T. N., Tande, C. A., Tenuta, A. U., A.Wise, K., and Wrather, A. J. 2017. Soybean yield loss estimates due to diseases in the United States and Ontario, Canada, from 2010 to 2014. Plant Health Progress18(1): 19-27.

6. Berglund, D. R. 2002. Soybean Production Field Guide for North Dakota and Northwestern Minnesota. Published in cooperative and with support from the North Dakota Soybean Council 136p.

7. Leandro, L. F., Robertson, A. E., Mueller, D. S., and Yang, X. B. 2013. Climatic and environmental trends observed during epidemic and non-epidemic years of soybean sudden death syndrome in Iowa. Plant Health Progress 14(1): 18.

8. Roy, K. W., Rupe, J. C., Hershman, D. E., and Abney, T. S. 1997. Sudden death syndrome of soybean. Plant Disease 81:1100-1111.

9. Yang, X. B. 1994. First report of sudden death syndrome of soybean in Iowa. Plant Disease 78:830.

10. Jardine, D. J. 1993. First report of sudden death syndrome of soybeans caused by *Fusarium solani* in Kansas. Plant Disease 77:1264.

11. Singh, R., Price, T., Padgett, B. and Burks, T. 2015. First report of sudden death syndrome of soybean caused by *Fusarium virguliforme* in Louisiana . Plant Health Progress 16:163–164.

12. Chilvers, M. I. and Brown-Rytlewski, D. E. 2010. First report and confirmed distribution of soybean sudden death syndrome caused by *Fusarium virguliforme* in Southern Michigan. Plant Disease 94: 1164–1164.

13. Ziems, A. D., Giesler, L. J. and Yuen, G. Y. 2006. First report of sudden death syndrome of soybean caused by *Fusarium solani* f. sp. *glycines* in Nebraska . Plant Disease 90: 109–109.

14. Cummings, J. A., Myers, K. L. and Bergstrom, G. C. 2018. First report of sudden death syndrome of soybean caused by *Fusarium virguliforme* in New York. Plant Disease 102:2036.

15. Nelson, B. D., Wilkinson, A. S., Markell, S. and Langseth, C. 2019. First report of sudden death syndrome of soybean caused by *Fusarium virguliforme* in North Dakota. Plant Disease 104:581.

16. Pennypacker, B. W. 1999. First report of sudden death syndrome caused by *Fusarium solani* f. sp. *glycines* on soybean in Pennsylvania . Plant Disease 83:879–879.

17. Tande, C., Hadi, B., Chowdhury, R., Subramanian, S. and Byamukama, E. 2014. First report of sudden death syndrome of soybean caused by *Fusarium virguliforme* in South Dakota. Plant Disease 98: 1012.

18. Bernstein, E. R., Atallah, Z. K., Koval, N. C., Hudelson, B. D. and Grau, C. R. 2007. First report of sudden death syndrome of soybean in Wisconsin. Plant Disease 91: 1201–1201.

19. Anderson, T. R. and Tenuta, A. U. 1998. First report of *Fusarium solani* f. sp. *glycines* causing sudden death syndrome of soybean in Canada. Plant Disease 82: 448.

20. Kurle, J. E., Gould, S. L., Lewandowski, S. M., Li, S. and Yang, X. B. 2003. First report of sudden death syndrome (*Fusarium solani* f. sp. *glycines*) of soybean in Minnesota . Plant Disease 87: 449–449.

21. Aoki, T., O'Donnell, K. and Scandiani, M. M. 2005. Sudden death syndrome of soybean in South America is caused by four species of Fusarium: *Fusarium brasiliense* sp. nov., *F. cuneirostrum* sp. nov., *F. tucumaniae*, and *F. virguliforme*. Mycoscience 46: 162–183.

22. Tewoldemedhin, Y. T., Lamprecht, S. C., Geldenhuys, J. J. and Kloppers, F. J. 2014. First report of soybean sudden death syndrome caused by *Fusarium virguliforme* in South Africa. Plant Disease 98: 569.

23. Chehri, K., Salleh, B., and Zakaria, L. 2014. *Fusarium virguliforme*, a soybean sudden death syndrome fungus in Malaysian soil. Australasian Plant Disease Notes 9(1), 128.

24. Roy, K. W. 1997. *Fusarium solani* on soybean roots: Nomenclature of the causal agent of sudden death syndrome and identity and relevance of *F. solani* form B. Plant Disease 81: 259–266.

25. Aoki, T., Tanaka, F., Suga, H., Hyakumachi, M., Scandiani, M.M. and O'Donnell, K. 2012. *Fusarium azukicola* sp. nov., an exotic azuki bean root-rot pathogen in Hokkaido, Japan. Mycologia 104 (5): 1068–1084.

26. Aoki, T., Scandiani, M. M. and O'Donnell, K. 2012. Phenotypic, molecular phylogenetic, and pathogenetic characterization of *Fusarium crassistipitatum* sp. nov., a novel soybean sudden death syndrome pathogen from Argentina and Brazil. Mycoscience 53: 167–186.

27. Aoki, T., O'Donnell, K., Homma, Y. and Lattanzi, A. R. 2003. Sudden-death syndrome of soybean is caused by two morphologically and phylogenetically distinct species within the *Fusarium solani* species complex - *F. virguliforme* in North America and *F. tucumaniae* in South America. Mycologia 95: 660-684.

28. Wang, J., Sang, H., Jacobs, J. L., Oudman, K. A., Hanson, L. E., and Chilvers, M. I. 2019. Soybean sudden death syndrome causal agent *Fusarium brasiliense* present in Michigan. Plant Disease 103(6): 1234–1243.

29. Hartman, G. L., Chang, H. X. and Leandro, L. F. 2015. Research advances and management of soybean sudden death syndrome. Crop Protection 73: 60–66.

30. Brar, H. K., Swaminathan, S. and Bhattacharyya, M. K. 2011. The *Fusarium virguliforme* toxin FvTox1 causes foliar sudden death syndrome-like symptoms in soybean. Molecular Plant-Microbe Interactions 24: 1179–1188.

31. Abdelsamad, N. A., Baumbach, J., Bhattacharyya, M. K. and Leandro, L. F. 2017. Soybean sudden death syndrome caused by *Fusarium virguliforme* is impaired by prolonged flooding and anaerobic conditions. Plant Disease 101: 712–719.

32. Farias Neto, A. F., Hashmi, R., Schmidt, M., Carlson, S. R., Hartman, G. L., Li, S., Nelson, R. L., and Diers, B. W. 2007. Mapping and confirmation of a new sudden death syndrome resistance QTL on linkage group D2 from the soybean genotypes PI 567374 and 'Ripley'. Molecular Plant Breeding 20:53- 62.

33. Kandel, Y. R., Wise, K. A., Bradley, C. A., Chilvers, M. I., Tenuta, A. U., and Mueller, D. S. 2016. Fungicide and cultivar effects on sudden death syndrome and yield of soybean. Plant Disease 100(7): 1339–1350.

34. Wang, J., Bradley, C. A., Stenzel, O., Pedersen, D. K., Reuter-Carlson, U., and Chilvers, M. I. 2017. Baseline Sensitivity of *Fusarium virguliforme* to fluopyram fungicide. Plant Disease 101(4): 576–582.

35. McLean, K. S. and Lawrence, G. W. 1993. Interrelationship of *Heterodera glycines* and *Fusarium solani* in sudden death syndrome of soybean. Journal of Nematology 25: 434–439.

36. Melgar, J., Roy, K. W. and Abney, T. S. 1994. Sudden death syndrome of soybean: Etiology, symptomatology, and effects of irrigation and *Heterodera glycines* on incidence and severity under field conditions. Canadian Journal of Botany 72: 1647–1653.

37. Westphal A, Li C, Xing L, McKay A, Malvick D. 2014. Contributions of *Fusarium virguliforme* and *Heterodera glycines* to the disease complex of sudden death syndrome of soybean. PLOS ONE 9(6): e99529

38. Kolander, T. M., Bienapfl, J. C., Kurle, J. E. and Malvick, D. K. 2012. Symptomatic and asymptomatic host range of *Fusarium virguliforme*, the causal agent of soybean sudden death syndrome. Plant Disease 96: 1148–1153.

39. Luo, Y., Chong, S. K. and Myers, O. 2001. Spatio-temporal analysis of soybean root colonization by *Fusarium solani* f. sp. *glycines* in fields. Plant Disease 85: 303–310.

40. Kandel, Y. R., Wise, K. A., Bradley, C. A., Tenuta, A. U. and Mueller, D. S. 2016. Effect of planting date, seed treatment, and cultivar on plant population, sudden death syndrome, and yield of Soybean. Plant Disease 100: 1735–1743.

41. Bao, Y., J.E. Kurle, G. Anderson, and N.D. Young. 2015. Association mapping and genomic prediction for resistance to sudden death syndrome in early maturing soybean germplasm. Molecular Breeding 35:128.

42. Chang, H., M.G. Roth, D. Wang, S.R. Cianzio, D.A. Lightfoot, G.L. Hartman, and M.I. Chilvers. 2018. Integration of sudden death syndrome resistance loci in the soybean genome. Theoretical Applied Genetics 131:757–773.

43. Weems, J. D., Haudenshield, J. S., Bond, J. P., Hartman, G. L., Ames, K. A., and Bradley, C. A. 2015. Effect of fungicide seed treatments on *Fusarium virguliforme* infection of soybean and development of sudden death syndrome. . Canadian Journal of Plant Pathology 37: 435–447

44. Sierotzki, H. and Scalliet, G. 2013. A review of current knowledge of resistance aspects for the next-generation succinate dehydrogenase inhibitor fungicides. Phytopathology 103: 880–887.

45. Xing, L. and Westphal, A. 2006. Interaction of *Fusarium solani* f. sp. *glycines* and *Heterodera glycines* in sudden death syndrome of soybean. Phytopathology 96: 763–770.

46. Xing, L. and Westphal, A. 2013. Synergism in the interaction of *Fusarium virguliforme* with *Heterodera glycines* in sudden death syndrome of soybean. Journal of Plant Diseases and Protection 120: 209–217.

47. Gao, X., Jackson, T. A., Hartman, G. L. and Niblack, T. L. 2006. Interactions between the soybean cyst nematode and *Fusarium solani* f. sp. *glycines* based on greenhouse factorial experiments. Phytopathology 96: 1409–1415.

48. Marburger, D., Conley, S., Esker, P., Macguidwin, A. and Smith, D. 2014. Relationship between *Fusarium virguliforme* and *Heterodera glycines* in commercial soybean fields in Wisconsin. Plant Health Progress 15: 11–18.

49. Krupinsky, J. M., Bailey, K. L., McMullen, M. P., Gossen, B. D. and Turkington., T. K. 2002. Managing plant disease risk in diversified cropping systems. Agronomy Journal 94:198–209.

50. Wrather, J. A., Kendig, S. R., Anand, S. C., Niblack, T. L. and Smith, G. S. 1995. Effects of tillage, cultivar, and planting date on percentage of soybean leaves with symptoms of sudden death syndrome. Plant Disease 79: 560–562.

51. Scherm, H. and Yang, X. B. 1996. Development of sudden death syndrome of soybean in relation to soil temperature and soil water matric potential. Phytopathology 86: 642–649.

52. Scherm, H., Yang, X.B., and Lundeen, P. 1998 Soil variables associated with sudden death syndrome in soybean fields in Iowa. Plant Disease 82: 1152–1157.

53. Vick, C. M., Bond, J. P., Chong, S. K. and Russin, J. S. 2006. Response of soybean sudden death syndrome to tillage and cultivar. Canadian Journal of Plant Pathology. 28: 77–83.

54. Vick, C. M., Chong, S. K., Bond, J. P., and Russin, J. S. 2003. Response of soybean sudden death syndrome to subsoil tillage. Plant Disease 87(6): 629-632.

55. Chong, S. K., Hildebranda, K. K., Luob, Y., Myersa, O., Indorantec, S. J., Kazakeviciusd, A., Russina, J. 2005. Mapping soybean sudden death syndrome as related to yield and soil/site properties. Soil and Tillage Research 84: 101-107.

56. Bailey, K. L. and Lazarovits, G. 2003. Suppressing soil-borne diseases with residue management and organic amendments. Soil and Tillage Research 72: 169–180.

57. Leandro, L.F.S., Eggenberger, S., Chen, C., Williams, J., Beattie, G.A., and Liebman, M. 2018. Cropping system diversification reduces severity and incidence of soybean sudden death syndrome caused by *Fusarium virguliforme*. Plant Disease 102: 1748–1758.

58. Rupe, J. C., Robbins, R. T. and Gbur, E. E. 1997. Effect of crop rotation on soil population densities of *Fusarium solani* and *Heterodera glycines* and on the development of sudden death syndrome of soybean. Crop Protection 16: 575–580.

59. Xing, L. and Westphal, A. 2009. Effects of crop rotation of soybean with corn on severity of sudden death syndrome and population densities of *Heterodera glycines* in naturally infested soil. Field Crops Research 112: 107–117.

60. Navi, S. S., and Yang, X. B. 2016. Impact of crop residue and corn-soybean rotation on the survival of *Fusarium virguliforme* a causal agent of sudden death syndrome of soybean. Journal of Plant Pathology & Microbiology 7:330

61. Vosberg, S. K., Marburger, D. A., Smith, D. L. and Conley, S. P. 2017. Planting date and fluopyram seed treatment effect on soybean sudden death syndrome and seed yield. Agronomy Journal 109: 2570–2578.

62. Bienapfl, J. C. 2011. Fusarium and Phytophthora species associated with root rot of soybean *(Glycine max)* (PhD Thesis, University of Minnesota, Saint-Paul, MN. Retrived from http://hdl.handle.net/11299/101446.

63. Díaz Arias, M. M., Munkvold, G. P., Ellis, M. L. and Leandro, L. F. S. 2013. Distribution and frequency of *Fusarium* species associated with soybean roots in Iowa. Plant Disease 97:1557–1562.

64. Marburger, D.A., Smith, D. L., and Conley, S. P. 2019. Characterizing interactions between *Fusarium graminearum* and *Fusarium virguliforme* on early soybean growth and development. Canadian Journal of Plant Pathology 41(2): 251-260.

65. Kerr, A. 1963. The root rot–Fusarium wilt complex of peas. Australian Journal of Biological Sciences 16(1):55-69.

66. Pieczarka, D. J. and Abawi, G. S. 1978. Effect of interaction between *Fusarium, Pythium,* and *Rhizoctonia* on severity of bean root rot. Phytopathology 68: 403–408.

67. Garcia, R. and Mitchell, D. J. 1975. Synergistic Interactions of *Pythium myriotylum* with *Fusarium solani* and *Meloidogyne arenaria* in Pod Rot of Peanut. Phytopathology 65: 832-833.

68. Lerch-Olson, E. R., and Robertson, A. E. 2019. Effect of co-inoculations with Pythium and Fusarium species on seedling disease development of soybean. Canadian Journal of Plant Pathology 1-11.

69. Datnoff, L. E., and Sinclair, J. B. 1988. The interaction of *Fusarium oxysporum* and *Rhizoctonia solani* in causing root rot of soybean. Phytopathology 78:771-777.

70. Rossman, A.Y., Seifert, K.A., Samuels, G.J., Minnis, A.W., Schroers, H.J., Lombard, L., Crous, P.W., Poldmaa, K., Cannon, P.F., Summerbell, R.C., Geiser, D.M., Zhuang, W., Hirooka, Y., Herrera, C., Salgado-Salazar, C. and Chaverri, P. 2013. Genera in Bionectriaceae, Hypocreaceae, and Nectriaceae (Hypocreales) proposed for acceptance or rejection. IMA Fungus, 4(1): 41-51.

71. Jensen, B., Knudsen, I. M. B. and Jensen, D. F. 2000. Biological seed treatment of cereals with fresh and long-term stored formulations of *Clonostachys rosea*: biocontrol efficacy against *Fusarium culmorum*. European Journal of Plant Pathology 106: 233–242.

72. Xue, A., Chen, Y., Voldeng, H., Savard, M., and Tian, X. 2008. Biological control of Fusarium head blight of wheat with Clonostachys rosea strain ACM941. Cereal Research Communications 36(Supplement-6), 695-699.

73. Chatterjee, S., Kuang, Y., Splivallo, R., Chatterjee, P. and Karlovsky, P. 2016. Interactions among filamentous fungi *Aspergillus Niger, Fusarium verticillioides* and *Clonostachys rosea*: Fungal biomass, diversity of secreted metabolites and fumonisin production. BMC Microbiology 16: 83.

74. Luongo, L., Galli, M., Corazza, L., Meekes, E., De Haas, L., Van der Plas, C.L. and Kohl, J. 2005. Potential of fungal antagonists for biocontrol of Fusarium spp. in wheat and maize through competition in crop debris. Biocontrol Science and Technology 15: 229–242.

75. Bienapfl, J. C., Floyd, C. M., Percich, J. A. and Malvick, D. K. First report of *Clonostachys rosea* causing root rot of soybean in the United States. Plant Disease 96:1700.

76. Afshari, N. and Hemmati, R. 2017. First report of the occurrence and pathogenicity of *Clonostachys rosea* on faba bean. Australasian Plant Pathology 46: 231–234.

77. Coleman, J. J. 2016. The *Fusarium solani* species complex: Ubiquitous pathogens of agricultural importance. Molecular Plant Pathology17: 146–158.

78. Michielse, C. B. and Rep, M. 2009. Pathogen profile update: *Fusarium oxysporum.* Molecular Plant Patholology 10: 311–324.

79. Le May, C., Potage, G., Andrivon, D., Tivoli, B. and Outreman, Y. 2009. Plant disease complex: Antagonism and synergism between pathogens of the ascochyta blight complex on pea. Journal of Phytopathology 157: 715–721.

80. Abdullah, A. S., Moffat, C. S., Lopez-Ruiz, F. J., Gibberd, M. R., Hamblin, J., and Zerihun, A. 2017. Host-multi-pathogen warfare: Pathogen interactions in co-infected plants. Frontiers in plant science 8:1806.

81. Miedaner T, Schilling AG. 1996. Genetic variation of aggressiveness in individual field populations of *Fusarium graminearum* and *Fusarium culmorum* tested on young plants of winter rye. European Journal of Plant Pathology 102:823–830.

82. Reid, L.M., Nicol, R.W., Ouellet, T., Savard, M., Miller, J.D., Young, J.C., Stewart, D.W., and Schaafsma, A.W. 1999. Interaction of *Fusarium graminearum* and *F. moniliforme* in maize ears: Disease progress, fungal biomass, and mycotoxin accumulation. Phytopathology 89:1028-1037.

83. Pugh, G. J. F., Wicklow, D. T. and Carroll, G. C. 1983. The fungal community. its organization and role in the ecosystem. Mycologia 75: 571.

84. Jonkers, W., Rodriguez Estrada, A., Lee, K., Breakspear, A., May, G., and Kistler, H. 2012. Metabolome and transcriptome of the interaction between *Ustilago maydis* and *Fusarium verticillioides* in vitro. Applied and environmental microbiology 78:3656–3637.

85. Mousa, W. K., Schwan, A., Davidson, J., Strange, P., Liu, H., Zhou, T., Auzanneau, F. I., and Raizada, M. N. 2015. An endophytic fungus isolated from finger millet (*Eleusine coracana*) produces anti-fungal natural products. Frontiers in microbiology, 6:1157.

86. Mousa, W. K., Shearer, C., Limay-Rios, V., Ettinger, C. L., Eisen, J. A., and Raizada, M. N. 2016. Root-hair endophyte stacking in finger millet creates a physicochemical barrier to trap the fungal pathogen *Fusarium graminearum.* Nature microbiology 1:16167.

87. Rheeder, J. P., Marasas, W. F. O. and Van Wyk, P. S. 1990. Fungal associations in corn kernels and effects on germination. Phytopathology. 80:131–134.

88. Xu, X. M., Monger, W., Ritieni, A. and Nicholson, P. 2007. Effect of temperature and duration of wetness during initial infection periods on disease development, fungal biomass and mycotoxin concentrations on wheat inoculated with single, or combinations of *Fusarium* species. Plant Pathology 56: 943–956.

89. Aimé, S., Alabouvette, C., Steinberg, C. and Olivain, C. 2013. The endophytic strain *Fusarium oxysporum* Fo47: A good candidate for priming the defense responses in tomato roots. Molecular Plant-Microbe Interactions 26: 918–926.

90. Kuzdraliński, A.,Szczerba, H.,Tofil, K., Filipiak, A.,Garbarczyk, E., Dziadko, Muszyńska, M., and Solarska. E. 2014. Early PCR-based detection of *Fusarium culmorum, F. graminearum, F. sporotrichioides* and *F. poae* on stem bases of winter wheat throughout Poland. European Journal of Plant Pathology 140:491–502.

91. Picot, A., Hourcade-Marcolla, D., Barreau, C., Pinson-Gadais, L., Caron, D., Richard-Forget, F. and Lannou, C. 2012. Interactions between *Fusarium verticillioides* and *Fusarium graminearum* in maize ears and consequences for fungal development and mycotoxin accumulation. Plant Pathology 61:140-151.

92. Saunders, M. and Kohn, L. M. 2008. Host-synthesized secondary compounds influence the in vitro interactions between fungal endophytes of maize. Applied and environmental microbiology 74:136–142.

93. Leslie, J. F. and Summerell, B. A. 2006. The Fusarium Laboratory Manual. Blackwell Publishing, Hoboken, 1-2.

94. Bretag, T. W. 1985. Fungi associated with root rots of annual *Medicago* spp. in Australia. Transactions of the British Mycological Society 85: 329–334.

95. Zitnick-Anderson, K., Simons, K. and Pasche, J. S. 2018. Detection and qPCR quantification of seven *Fusarium* species associated with the root rot complex in field pea. Canadian Journal of Plant Patholoy 40: 261–271.

96. Nik, W. Z. and Parbery, D. G. 1977. Studies of seed-borne fungi of tropical pasture legume species. Australian Journal of Agricultural Research 28: 821–841.

97. Mao, W., Carroll, R. B. and Whittington, D. P. 1998. Association of *Phoma terrestris, Pythium irregulare,* and *Fusarium acuminatum* in causing red root rot of corn. Plant Disease 82: 337–342.

98. Gonzalez, M. S. and Trevathan, L. E. 2000. Identity and pathogenicity of fungi associated with root and crown rot of soft red winter wheat grown on the Upper Coastal Plain Land Resource Area of Mississippi. Journal of Phytopathology 148: 77–85.

99. Strausbaugh, C. A., Overturf, K. and Koehn, A. C. 2005. Pathogenicity and real-time PCR detection of *Fusarium* spp. in wheat and barley roots. Canadian Journal of Plant Pathology 27(3): 430-438.

100. Wang, Y., Gua, Y. M., Lu, B. H. and Gao, J. 2016. First report of ginseng (*Panax ginseng*) root rot caused by *Fusarium acuminatum* in China. Plant Disease 100:525.

101. Elmer, W. H. 1996. *Fusarium* fruit rot of pumpkin in Connecticut. Plant Disease 80: 131–135.

102. Wong, D. H., Barbett, M. J. and Sivasithamparam, K. 1985. Fungi Associated With Root Rot of Subterranean Clover in Western Australia. Australian Journal of Experimental Agriculture 25: 574-579.

103. Hanson, L. E. and Hill, A. L. 2004. *Fusarium* species causing Fusarium yellows of sugarbeet. Journal of Sugarbeet Research 41:163-178.

104. Edel-Hermann, V. and Lecomte, C. 2019. Current status of fusarium oxysporum formae speciales and races. Phytopathology 109(4): 512–530.

105. Correll, J. C. 1991. The relationship between formae speciales, races, and vegetative compatibility groups in *Fusarium oxysporum*. Phytopathology 81:1061–1064 (1991).

106. Olivain, C. and Alabouvette, C. 1999. Process of tomato root colonization by a pathogenic strain of *Fusarium oxysporum* f. sp. *lycopersici* in comparison with a non-pathogenic strain. The New Phytologist 141:497–510.

107. Zhang, J. X., Xue, A. G., Zhang, H. J., Nagasawa, A. E. and Tambong, J. T. 2010. Response of soybean cultivars to root rot caused by *Fusarium* species. Canadian Journal of Plant Science 90:767-776.

108. Alleman, R. J., Grau, C. R., and Hogg, D. B. 2002. Soybean aphid host range and virus transmission efficiency. In *Proceedings of Wisconsin Fertilizer, Aglime, Pest Manage. Conference,* Madison, WI. (http://alfi.soils.wisc.edu/extension/wcmc/2002proceedings/Alleman-Conf-2002.pdf.)

109. Tilmon, K. J., Hodgson, E. W., O'Neal, M. E. and Ragsdale, D. W. 2011. Biology of the Soybean Aphid, *Aphis glycines* (Hemiptera: Aphididae) in the United States. Journal of Integrated Pest Management 2:1-7.

110. Hunt, D., Foottit, R., Gagnier, D. and Baute, T. 2003. First Canadian records of *Aphis glycines* (Hemiptera: Aphididae). Canadian Entomologist 135: 879–881.

111. Ragsdale, D. W., Voegtlin, D. J. and O'Neil, R. J. 2004. Soybean aphid biology in North America. *Ann. Entomol. Soc. Am.* 97: 204–208.

112. Rutledge, C. E. 2005. First record of the soybean aphid, *Aphis glycines* (Hemiptera: Sternorrhyncha: Aphididae) in Connecticut and Massachusetts *The* Great Lakes Entomologist 37:198–201.

113. Ragsdale, D. W., Landis, D. A., Brodeur, J., Heimpel, G. E. and Desneux, N. 2011. Ecology and management of the soybean aphid in North America. Annual

review of entomology 56: 375–399.

114. USDA-NASS. 2019. NASS 2018 Agricultural Chemical Use Survey - Soybean. Report No. 2019-3.

115. Wu, Z., Schenk-Hamlin, D., Zhan, W., Ragsdale, D. W. and Heimpel, G. E. 2004. The soybean aphid in China: A historical review. Annals of the Entomological Society of America 97: 209–218.

116. Macedo, T. B., Bastos, C. S., Higley, L. G., Ostlie, K. R., and Madhavan, S. 2003. Photosynthetic responses of soybean to soybean aphid (Homoptera: Aphididae) injury. Journal of economic entomology 96(1): 188–193.

117. Clark, A. J. and Perry, K. L. 2002. Transmissibility of field isolates of soybean viruses by *Aphis glycines*. Plant Disease 86: 1219–1222.

118. Wang, R. Y. and Ghabrial, S. A. 2002. Effect of aphid behavior on efficiency of transmission of soybean mosaic virus by the soybean-colonizing aphid, *Aphis glycines*. Plant Disease 86: 1260–1264.

119. Dedryver, C. A., Le Ralec, A. and Fabre, F. 2010. The conflicting relationships between aphids and men: A review of aphid damage and control strategies. Comptes Rendus Biologies *3*33, 539–553.

120. Ragsdale, D. W., McCornack, B. P., Venette, R. C., Potter, B. D., MacRae, I. V., Hodgson, E. W., O'Neal, M. E., Johnson, K. D., O'Neil, R. J., DiFonzo, C. D., Hunt, T. E., Glogoza, P. A., and Cullen, E. M. 2007. Economic threshold for soybean aphid (Hemiptera: Aphididae). Journal of economic entomology 100(4): 1258–1267

121. Beckendorf, E. A., Catangui, M. A. and Riedell, W. E. 2008. Soybean aphid feeding injury and soybean yield, yield components, and seed composition. Agronomy Journal 100: 237–246.

122. Hodgson, E. W., Venette, R. C., Abrahamson, M. and Ragsdale, D. W. 2009. Alate production of soybean aphid (Homoptera: Aphididae) in Minnesota. Environmental Entomology 34: 1456–1463.

123. Müller, C. B., Williams, I. S. and Hardie, J. 2001. The role of nutrition, crowding and interspecific interactions in the development of winged aphids Ecological Entomology 26: 330–340.

124. Ríos Martínez, A. F., & Costamagna, A. C. 2018. Effects of crowding and host plant quality on morph determination in the soybean aphid, *Aphis glycines*. Entomologia Experimentalis et Applicata 166(1): 53-62.

125. Bahlai, C. A., Welsman, J. A., Schaafsma, A. W. and Sears, M. K. 2007. Development of soybean aphid (Homoptera: Aphididae) on its primary overwintering host, *Rhamnus cathartica*. Environmental Entomology 36: 998–1006.

126. Steffey, K., & Gray, M. 2012. Managing insect pests. *Illinois agronomy handbook* 179-196.

127. Koch, R. L., Hodgson, E. W., Knodel, J. J., Varenhorst, A. J., and Potter, B. D. 2018. Management of insecticide-resistant soybean aphids in the Upper Midwest of the United States. Journal of Integrated Pest Management 9(1): 23.

128. Johnson, K. D., O'Neal, M. E., Ragsdale, D. W., DiFonzo, C. D., Swinton, S. M., Dixon, P. M., Potter, B. D., Hodgson, E. W., and Costamagna, A. C. 2009. Probability of cost-effective management of soybean aphid (Hemiptera: Aphididae) in North America. Journal of economic entomology 102(6): 2101–2108.

129. Koch, R.L., Potter, B.D., Glogoza, P.A., Hodgson, E.W., Krupke, C.H., Tooker, J.F., DiFonzo, C.D., Michel, A.P., Tilmon, K.J., Prochaska, T.J. and Knodel, J.J. 2016. Biology and economics of recommendations for insecticide-based management of soybean aphid. Plant Health Progress 17(4): 265-269.

130. Wang, Q., Xu, W. J., Yan, S. C., and Wang, C. 2011. Research on Resistance of Aphis Glycines in Hei Longjiang Province. Advanced Materials Research 393–395: 926–929.

131. Hanson, A. A., Menger-Anderson, J., Silverstein, C., Potter, B. D., MacRae, I. V., Hodgson, E. W., and Koch, R. L. 2017. Evidence for soybean aphid (Hemiptera: Aphididae) resistance to pyrethroid insecticides in the upper midwestern United States. Journal of economic entomology 110(5): 2235-2246.

132. Brewer, M. J. and Noma, T. 2010. Habitat affinity of resident natural enemies of the invasive *Aphis glycines* (Hemiptera: Aphididae), on soybean, with comments on biological control. Journal of economic entomology. 103: 583–596.

133. Nielsen, C. and Hajek, A. E. 2006. Control of invasive soybean aphid, *Aphis glycines* (Hemiptera: Aphididae), populations by existing natural enemies in new york state, with emphasis on entomopathogenic fungi. Environmental Entomology 34:1036–1047.

134. Rutledge, C. E., O'Neil, R. J., Fox, T. B. and Landis, D. A. 2004. Soybean aphid predators and their use in integrated pest management. Annals of the Entomological Society of America 97: 240–248.

135. Koch, K. A., Potter, B. D. and Ragsdale, D. W. 2010. Non-target impacts of soybean rust fungicides on the fungal entomopathogens of soybean aphid ournal of invertebrate pathology 103: 156–164.

136. Li, Y., Hill, C. B. and Hartman, G. L. 2004. Effect of three resistant soybean genotypes on the fecundity, mortality, and maturation of soybean aphid (Homoptera:Aphididae). Journal of Economic Entomology 97: 1106–1111.

137. Mian, M. A. R., Hammond, R. B. and St Martin, S. K. 2008. New plant introductions with resistance to the soybean aphid. Crop Science 48:1055–1061.

138. Zhang, G., Gu, C. and Wang, D. 2009. Molecular mapping of soybean aphid resistance genes in PI 567541B. Theorectical Applied Genetics 118: 473–482.

139. Hill, C. B., Crull, L., Herman, T. K., Voegtlin, D. J. and Hartman, G. L. 2010. A new soybean aphid (Hemiptera: Aphididae) biotype identified. Journal of Economic Entomology 103: 509–515.

140. Kim, K. S., Hill, C. B., Hartman, G. L., Rouf Mian, M. A. and Diers, B. W. 2008. Discovery of soybean aphid biotypes. Crop Science 48: 923–928.

141. Stout, M. J., Thaler, J. S. and Thomma, B. P. H. J. 2006. Plant-Mediated interactions between pathogenic microorganisms and herbivorous arthropods. Annual Review of Entomology 51: 663–689.

142. Gupta, S., Krasnoff, S. B., Underwood, N. L., Renwick, J. A. A., and Roberts, D. W. 1991. Isolation of beauvericin as an insect toxin from *Fusarium semitectum* and *Fusarium moniliforme* var. *subglutinans*. Mycopathologia 115(3): 185-189.

143. Tack, A. J. M. and Dicke, M. 2013. Plant pathogens structure arthropod communities across multiple spatial and temporal scales. Functional Ecology 27: 633–645.

144. Castagneyrol, B., Fernandez-Conradi, P., Rasmussen, P. U., Robin, C., and Tack, A. J. 2018. Belowground–aboveground interactions between pathogens and herbivores. Pages 135-174 in: Aboveground–Belowground Community Ecology. Ohgushi, T., Wurst, S., and Johnson, S. eds. Springer, Cham.

145. Notaguchi, M. and Okamoto, S. 2015. Dynamics of long-distance signaling via plant vascular tissues. Frontiers in Plant Science 6:161.

146. Biere, A. and Goverse, A. 2016. Plant-mediated systemic interactions between pathogens, parasitic nematodes, and herbivores above- and belowground. Annual Review of Phytopathology. 54: 499–527.

147. Wondafrash, M., Van Dam, N. M. and Tytgat, T. O. G. 2013. Plant systemic induced responses mediate interactions between root parasitic nematodes and aboveground herbivorous insects. Frontiers in Plant Science 4: 87.

148. Barber, N. A., Milano, N. J., Kiers, E. T., Theis, N., Bartolo, V., Hazzard, R. V., and Adler, L. S. 2015. Root herbivory indirectly affects above-and below-ground community members and directly reduces plant performance. Journal of Ecology 103(6): 1509-1518.

149. Lee, B., Lee, S. and Ryu, C. M. 2012. Foliar aphid feeding recruits rhizosphere bacteria and primes plant immunity against pathogenic and non-pathogenic bacteria in pepper. Annals of Botany 110: 281–290.

150. Pieterse, C. M. J. and Van Loon, L. C. 1999. Salicylic acid-independent plant defence pathways. Trends in Plant Science 4: 52–58.

151. Thaler, J. S., Humphrey, P. T. and Whiteman, N. K. 2012. Evolution of jasmonate and salicylate signal crosstalk. Trends in Plant Science 17: 260-270.

152. Spoel, S.H., Koornneef, A., Claessens, S.M., Korzelius, J.P., Van Pelt, J.A., Mueller, M.J., Buchala, A.J., Métraux, J.P., Brown, R., Kazan, K. and Van Loon, L.C. 2003. NPR1 modulates cross-talk between salicylate- and jasmonate-dependent defense pathways through a novel function in the cytosol. The Plant Cell 15: 760–770.

153. Koornneef, A., Leon-Reyes, A., Ritsema, T., Verhage, A., Den Otter, F. C., Van Loon, L. C., and Pieterse, C. M. 2008. Kinetics of salicylate-mediated suppression of jasmonate signaling reveal a role for redox modulation. Plant Physiology 147(3):1358-1368.

154. Yoshida, Y., Sano, R., Wada, T., Takabayashi, J. and Okada, K. 2009. Jasmonic acid control of GLABRA3 links inducible defense and trichome patterning in Arabidopsis. Development 136: 1039–1048.

155. Bezemer, T. M., Wagenaar, R., Van Dam, N. M., Van Der Putten, W. H. and Wäckers, F. L. 2004. Above- and below-ground terpenoid aldehyde induction in cotton, *Gossypium herbaceum*, following root and leaf injury. Journal of Chemical Ecology 30(1): 53-67.

156. Cardoza, Y. J., Teal, P. E. A. and Tumlinson, J. H. 2003. Effect of peanut plant fungal infection on oviposition preference by *Spodoptera exigua* and on host-searching behavior by *Cotesia marginiventris.* Environmental Entomology 32(5): 970-976.

157. Schwachtje, J. and Baldwin, I. T. 2008. Why does herbivore attack reconfigure primary metabolism? Plant Physiology 146: 845–851.

158. Moellenbeck, D. J., Quisenberry, S. S. and Colyer, P. D. 1992. Fusarium crown-rot development in alfalfa stressed by threecornered alfalfa hopper (Homoptera: Membracidae) feeding. Journal of Economic Entomology 85(4): 1442-1449.

159. Kostenko, O., van de Voorde, T. F. J., Mulder, P. P. J., van der Putten, W. H. and Martijn Bezemer, T. 2012. Legacy effects of aboveground-belowground interactions. Ecology Letters 15: 813–821.

160. Bezemer, T. M., De Deyn, G. B., Bossinga, T. M., Van Dam, N. M., Harvey, J. A., and Van der Putten, W. H. 2005. Soil community composition drives aboveground plant–herbivore–parasitoid interactions. Ecology Letters 8(6): 652-661.

161. Johnson, S. N., Douglas, A. E., Woodward, S. and Hartley, S. E. 2003. Microbial impacts on plant-herbivore interactions: The indirect effects of a birch pathogen on a birch aphid. Oecologia 134: 388–396.

162. Chiozza, M. V, O'Neal, M. E. and MacIntosh, G. C. 2010. Constitutive and induced differential accumulation of amino acid in leaves of susceptible and resistant soybean plants in response to the soybean aphid (Hemiptera: Aphididae). Environmental Entomology 39: 856–864.

163. Biere, A. and Honders, S. C. 2006. Coping with third parties in a nursery pollination mutualism: *Hadena bicruri*s avoids oviposition on pathogen-infected, less rewarding *Silene latifolia*. New Phytologist 169: 719–727.

164. Tack, A. J. M., Gripenberg, S. and Roslin, T. 2012. Cross-kingdom interactions matter: Fungal-mediated interactions structure an insect community on oak. Ecology Letters 15: 177–185.

165. Desurmont, G. A., Xu, H. and Turlings, T. C. J. 2016. Powdery mildew suppresses herbivore-induced plant volatiles and interferes with parasitoid attraction in *Brassica rapa*. Plant, Cell & Environment 39: 1920–1927.

166. Malvick, D. K. and Bussey, K. E. 2008. Comparative analysis and characterization of the soybean sudden death syndrome pathogen *Fusarium virguliforme* in the northern United States. Canadian Journal of Plant Pathology 30(3): 467-476.

167. Malvick, D. K. and Grunden, E. 2005. Isolation of fungal DNA from plant tissues and removal of DNA amplification inhibitors. Molecular Ecology Notes 5: 958–960.

168. Geiser, D.M., del Mar Jiménez-Gasco, M., Kang, S., Makalowska, I., Veeraraghavan, N., Ward, T.J., Zhang, N., Kuldau, G.A. and O'donnell, K., 2004. FUSARIUM-ID v. 1.0: a DNA sequence database for identifying Fusarium. European Journal of Plant Pathology 110(5-6), 473-479.

169. White, T. J., Bruns, T., Lee, S. J. W. T., and Taylor, J. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. PCR protocols: a guide to methods and applications 18(1), 315-322.

170. Kandel, Y.R., Haudenshield, J.S., Srour, A.Y., Islam, K.T., Fakhoury, A.M., Santos, P., Wang, J., Chilvers, M.I., Hartman, G.L., Malvick, D.K. and Floyd, C.M. 2015. Multilaboratory comparison of quantitative PCR assays for detection and quantification of *Fusarium virguliforme* from soybean roots and soil. Phytopathology 105: 1601–1611.

171. Gongora-Canul, C. C. and Leandro, L. F. S. 2011. Effect of soil temperature and plant age at time of inoculation on progress of root rot and foliar symptoms of soybean sudden death syndrome. Plant Disease 95: 436–440.

172. Ji, J., Scott, M. P. and Bhattacharyya, M. K. 2006. Light is essential for degradation of ribulose-1,5-bisphosphate carboxylase-oxygenase large subunit during sudden death syndrome development in soybean. Plant Biology 8: 597–605.

173. Freed, G. M., Floyd, C. M. and Malvick, D. K. 2017. Effects of pathogen population levels and crop-derived nutrients on development of soybean sudden death syndrome and growth of *Fusarium virguliforme*. Plant Disease 101: 434–441.

174. Njiti, V. N., Shenaut, M. A., Suttner, R. J., Schmidt, M. E. and Gibson, P. T. 1998. Relationship between soybean sudden death syndrome disease measures and yield components in F6-derived lines. Crop Science 38: 673–678.

175. Luo, Y., Hildebrand, K., Chong, S. K., Myers, O. and Russin, J. S. 2000. Soybean yield loss to sudden death syndrome in relation to symptom expression and root colonization by *Fusarium solani* f. sp. *glycines*. Plant Disease 84(8): 914-920.

176. Kandel, Y.R., Bradley, C.A., Chilvers, M.I., Mathew, F.M., Tenuta, A.U., Smith, D.L., Wise, K.A. and Mueller, D.S. 2020. Relationship between sudden death syndrome caused by *Fusarium virguliforme* and soybean yield: a meta-analysis. Plant Disease PDIS-11.

177. Catangui, M. A., Beckendorf, E. A. and Riedell, W. E. 2009. Soybean aphid population dynamics, soybean yield loss, and development of stage-specific economic injury levels. Agronomy Journal 101: 1080–1092.

178. Riedell, W. E. and Catangui, M. A. 2006. Greenhouse studies of soybean aphid (Hemiptera: Aphididae) effects on plant growth, seed yield and composition. Journal of Agricultural and Urban Entomology 23: 225-235.

179. Abeysekara, N. S. and Bhattacharyya, M. K. 2014. Analyses of the xylem sap proteomes identified candidate *Fusarium virguliforme* proteinacious toxins. PLoS One 9(5): e93667.

180. Guerrieri, E. and Digilio, M. C. 2008. Aphid-plant interactions: a review. Journal of Plant Interactions 3: 223–232.

181. Karley, A. J., Douglas, A. E., and Parker, W. E. 2002. Amino acid composition and nutritional quality of potato leaf phloem sap for aphids. Journal of Experimental Biology 205(19): 3009-3018.

182. O'Donnell, K., Sink, S., Scandiani, M. M., Luque, A., Colletto, A., Biasoli, M., Lenzi, L., Salas, G., González, V., Ploper, L. D., Formento, N., Pioli, R. N., Aoki, T., Yang, X. B., and Sarver, B. A. 2010. Soybean sudden death syndrome species diversity within North and South America revealed by multilocus genotyping. Phytopathology 100(1): 58–71.

183. Hartman, G. L., Huang, Y. H., Nelson, R. L. and Noel, G. R. 1997. Germplasm evaluation of Glycine max for resistance to *Fusarium solani,* the causal organism of sudden death syndrome. Plant Disease 81(5), 515-518.

184. Menger, J., Beauzay, P., Chirumamilla, A., Dierks, C., Gavloski, J., Glogoza, P., Hamilton, K., Hodgson, E.W., Knodel, J.J., MacRae, I.V. and Pezzini, D.T. 2020. Implementation of a Diagnostic-Concentration Bioassay for Detection of Susceptibility to Pyrethroids in Soybean Aphid (Hemiptera: Aphididae). Journal of Economic Entomology 113(2): 932-939.

185. Gibson, P.T., Shenaut, M.A., Njiti, V.J., Suttner, R.J., and Myers, O., J. 1994. Soybean varietal response to sudden death syndrome. Pages 6-7 in: Proc. 24th Soybean Seed Res Conf., Chicago, Illinois

186. Hanafi, A., Radcliffe, E. B. and Ragsdale, D. W. 1989. Spread and Control of Potato Leafroll Virus in Minnesota. Journal of Economic Entomology 82: 1201–1206.

187. Radwan, O., Liu, Y. and Clough, S. J. 2011. Transcriptional analysis of soybean root response to *Fusarium virguliforme*, the causal agent of sudden death syndrome. Molecular Plant-Microbe Interactions 24: 958–972.

188. Abeysekara, N. S., Swaminathan, S., Desai, N., Guo, L. and Bhattacharyya, M. K. 2016. The plant immunity inducer pipecolic acid accumulates in the xylem sap and leaves of soybean seedlings following *Fusarium virguliforme* infection. Plant Science 243: 105–114.

189. Hohenstein, J. D., Studham, M. E., Klein, A., Kovinich, N., Barry, K., Lee, Y. J., and MacIntosh, G. C.2019. Transcriptional and chemical changes in soybean leaves in response to long-term aphid colonization. Frontiers in Plant Science 10: 310.

190. Li, Y., Zou, J., Li, M., Bilgin, D. D., Vodkin, L. O., Hartman, G. L., and Clough, S. J. 2008. Soybean defense responses to the soybean aphid. New Phytologist 179(1): 185-195.

191. Mccornack, B. P., Ragsdale, D. W. and Venette, R. C. 2004. Demography of soybean aphid (Homoptera:Aphididae) at summer temperatures. Economic Entomology 97: 854–861.

192. Hong, S. C., Donaldson, J. and Gratton, C. 2010. Soybean Cyst Nematode Effects on Soybean Aphid Preference and Performance in the Laboratory. Environmental Entomology 39: 1561–1569.

193. Hong, S. C., Macguidwin, A. and Gratton, C. 2011. Soybean aphid and soybean cyst nematode interactions in the field and effects on soybean yield. Journal of Economic Entomology 104: 1568–1574.

194. Heeren, J. R., Steffey, K. L., Tinsley, N. A., Estes, R. E., Niblack, T. L., and Gray, M. E. 2012. The interaction of soybean aphids and soybean cyst nematodes on selected resistant and susceptible soybean lines. Journal of Applied Entomology 136(9): 646-655.

195. Mccarville, M. T., O'Neal, M., Tylka, G. L., Kanobe, C. and Macintosh, G. C. 2012. A nematode, fungus, and aphid interact via a shared host plant: Implications for soybean management. Entomologia Experimentalis et Applicata 143: 55–66.

196. McCarville, M. T., Soh, D. H., Tylka, G. L. and O'Neal, M. E. 2014. Aboveground feeding by soybean aphid, *Aphis glycines*, affects soybean cyst nematode, *Heterodera glycines,* reproduction belowground. PLoS One 9(1), e86415.

197. Costamagna, A. C. and Landis, D. A. 2006. Predators exert top-down control of soybean aphid across a gradient of agricultural management systems. Ecological Applications 16(4): 1619-1628.